SPATIOTEMPORAL PATTERNS OF DISTRIBUTION AND DRIVERS OF NEONICOTINOID INSECTICIDE FATE IN CANADIAN PRAIRIE POTHOLE WETLANDS

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ABSTRACT

Designed for the protection of major agricultural crops, neonicotinoids are the fastest-growing class of insecticides used against a broad spectrum of insect pests. Although neonicotinoid toxicity toward non-target organisms (e.g., bees, aquatic insects) has been well-studied, less is known about their distribution of use, transport, and fate in North American agroecosystems. This is especially true of neonicotinoid interactions with wetlands in the Canadian Prairies. Between 2009 and 2012, neonicotinoid use as a seed treatment increased by 30% across the Canadian Prairies. During spring 2012 to spring 2013, I sampled water and sediment from 136 wetlands situated in a range of crop types across central Saskatchewan to determine the extent of neonicotinoid contamination. Wetlands situated in oat, canola, and barley fields consistently contained higher neonicotinoid concentrations in water than in grasslands, but no single crop influenced overall detections. Neonicotinoid detections in water varied from 16% (fall 2012) to 91% (spring 2013) with peak concentrations up to 3110 ng/L found in summer. I found numerous detections of neonicotinoids in spring, after ice-off, but before seeding. Through sampling snow, snow meltwater, and soil particulates from previously treated (clothianidin) and untreated fields, meltwater showed the strongest relationship to initial spring concentrations in wetland water. Neonicotinoid concentrations increased with time in shallow temporary wetlands which appeared most at risk for annual contamination. While snowmelt contamination influenced water concentrations in spring, peak concentrations in wetlands were consistently found during summer sampling completed in 2012-2014. Rapid wetland assessments completed on 238 wetlands (summer of 2012 and 2013) revealed key ecological, hydrological and landscape features that influenced neonicotinoid detections and peak concentrations in Prairie wetlands. The results of my exploratory analysis indicated that plant community composition is a key indicator and/or driver of both detection and concentration of neonicotinoids in Prairie wetlands. In particular, specific shallow marsh plants were commonly associated with either higher (e.g., *Scirpus validus*) or lower (e.g., *Mentha arvensis*) neonicotinoid concentrations in natural wetlands suggesting wetland macrophytes in this zone may be either indicators of agricultural disturbance intensity or differentially capable of accumulating the insecticide in its tissue. Therefore, in 2014, I conducted an outdoor microcosm experiment to evaluate thiamethoxam uptake from water by *Typha latifolia* and *Alisma triviale* using two concentrations over a 7-day period. Experimental results found some trace positive detections but no

quantifiable accumulation of the insecticide in plant tissues. This is despite the fact that results of my 2015 field study found species of *Typha*, *Alisma* and *Equisetum* had neonicotinoids more frequently detected in their tissues, at concentrations ranging from 1.01-8.44 ug/kg. My findings demonstrate that neonicotinoid distribution and fate in Canadian Prairie agroecosystems is driven by interactions between ecological, hydrological, and landscape characteristics. Consequently, these drivers regulate neonicotinoid exposure and persistence in ecologically important regional wetlands. In order to effectively conserve these critical waterbodies, conservation planning should consider the importance of maintaining naturally diverse vegetation zones to mitigate insecticide exposure to wetland-dependant organisms.

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DEDICATION

I dedicate this dissertation to “armchair ecologists” everywhere; especially those of us who finally managed to get back into the field…

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LIST OF ABBREVIATIONS

AI	active ingredient
CLO	clothianidin
ha	hectare
IMI	imidacloprid
ng/L	nanograms per liter
ppb	parts per billion
PPR	Prairie Pothole Region
ppt	parts per trillion
SM	shallow marsh
THX	thiamethoxam
WM	wet meadow
µS/cm	micro Siemens per centimeter
µg/kg	micrograms per kilogram
µg/L	micrograms per liter

CHAPTER 1: INTRODUCTION

1.1 Research Purpose

Degradation of Canadian aquatic ecosystems from chemical inputs is a growing national concern because of the loss of ecosystem services provided through water supplies, habitat, and food resources for migratory birds, amphibians, and wildlife (Zedler and Kercher, 2005; Environment Canada, 2011). Globally, wetlands of the Prairie Pothole Region (PPR) are some of the most biologically diverse and productive ecosystems (Euliss *et al*.,2004). In agricultural areas, wetland water quality is threatened from expanding agricultural intensification by increased national reliance on agrochemicals (e.g., fertilizers, fungicides, herbicides and insecticides); and an increasingly heavy reliance on seed treatments as an application method (Environment Canada, 2011). Over half of this region's wetlands have historically been drained and the remaining intact wetlands are under stress due to sedimentation, tillage of marginal lands, and removal of surrounding vegetation communities as a result of agricultural activity (Kantrud and Newton, 1996; Bartzen *et al*., 2010).

Prairie wetlands occupy topographic depressions that naturally accumulate pesticides from deposition, spray-drift, and surface/subsurface runoff (Goldsborough and Crumpton, 1998). However, pesticide fate in Prairie wetlands is poorly understood as several features of wetlands (e.g., depth, emergent/submerged plant growth) may speed or slow dissipation of pesticides compared to that of other waterbodies (Goldsborough and Crumpton, 1998; Brogan and Relyea, 2014). Interactions between or among numerous physico-chemical properties (e.g., water solubility, log K_{OC}) coupled with environmental conditions need to be assessed to accurately determine pesticide fate in the wetland environment as no single factor can be used to predict pesticide behavior (Gavrilescu, 2005; Rice *et al*., 2007). Although most wetland classification and assessment systems examine many physical and biotic components of wetland health (Stewart and Kantrud, 1971; Guntenspergen *et al*., 2002; Fennessy *et al*., 2007), few examine the influence of surrounding land use and chemical stressors as part of their survey. As less is known about how pesticide fate is influenced by the interplay between abiotic (e.g., soil type, topography) and biotic factors (e.g., vegetation) at a range of spatial scales, studies of fate should be completed at both the local and landscape level (Beketov and Liess, 2012).

Current agricultural practices make intensive use of a newer class of insecticides, the neonicotinoids, which are widely used across the Canadian Prairies. Although valued for their versatility in application (Goulson, 2013), the majority are used in Canada as seed treatments on major agricultural crops such as canola, cereals (e.g., oat, wheat), corn, and pulses (Elbert *et al*., 2008). Currently, 80% of all treated seeds are coated using a neonicotinoid active ingredient (Jeschke *et al*., 2010), although most pesticide use and distribution data in Canada and the United States remains confidential (Main *et al*., 2014; Douglas and Tooker, 2015). Though neonicotinoids were originally designed to lessen the impact on the environment, in practice >90% of the active ingredient may enter surrounding soils (Goulson, 2013) where patterns of persistence can vary from a few days to years (PMRA, 2004; Jones *et al.*, 2014). Neonicotinoids are also highly water soluble; indeed, a range of concentrations have been detected in global surface water systems (Morrissey *et al*., 2015). To date, the specific fate of neonicotinoids in natural aquatic systems is poorly understood with knowledge gaps remaining as to why certain types of water bodies (e.g., wetlands) are more susceptible to contamination.

Ecological features such as wetland hydroperiod, connectivity, plant community composition, vegetation structure, and zonation typically affect biotic and abiotic factors of wetlands (Millar, 1976; van der Valk, 2012). Annual inundation and flooding by sources such as snowmelt, seasonal precipitation, and groundwater connectivity are directly linked to macroinvertebrate production, plant zonation, and vegetation composition (van der Kamp *et al*., 2003; Euliss *et al*., 2004; van der Kamp and Hayashi, 2009). However, these inputs may also increase the likelihood of neonicotinoid contamination in Prairie wetlands. As biotic and abiotic environmental factors can significantly affect the strength that persistent contaminants have on biological communities (Beketov and Liess, 2012), it is important to conduct intensive surveys across a large spatial scale to understand variation of pesticide distribution and fate (Johnson, 2002). Therefore, the purpose of this research is to explore the regional distribution of neonicotinoid use and how hydrological, ecological and landscape features may influence neonicotinoid insecticide fate in Canadian Prairie wetland ecosystems.

1.2 Research Objectives

By integrating wetland classification and assessment criteria with wetland ecotoxicological data, I explored the seasonal ecological, hydrological, and landscape features with the strongest

influence on neonicotinoid fate in Prairie wetlands. Working across a range of scales from the local (i.e., wetland) to the landscape level (i.e., PPR), the main objectives of this research were to:

- develop geospatial maps of current neonicotinoid use within the PPR in relation to annual crop rotations while surveying levels of neonicotinoids in water and sediment of a subset of wetlands surrounded by different crops through time;
- identify the major source or pathway of neonicotinoids to wetlands in spring and examine which factors affect changes in spring wetland neonicotinoid concentrations over time;
- determine the wetland and landscape features which may be most useful to predict neonicotinoid contamination;
- examine the ability of common wetland macrophytes to uptake and/or mitigate neonicotinoid residues entering surface waters.

1.3 Literature Review

This chapter provides background information on three themes that are central to this dissertation: Prairie wetlands, general pesticide use, and specifically, neonicotinoid insecticides. I start by describing Prairie wetland characteristics and functions, wetland classification, wetland assessment systems, and wetland stressors/wetland ecotoxicology. Next, I briefly explore some general information about pesticide use across the Canadian Prairies and the properties affecting pesticide fate and transport. Then, I give a brief background overview on neonicotinoid insecticides including their general use and toxicity, physico-chemical properties, and how these properties affect their fate in the environment. The chapter ends by a conclusion that summarizes some identified research gaps.

1.3.1 Prairie wetland characteristics and functions

The Canadian Wetland Classification System defines a wetland as "land that is saturated with water long enough to promote wetland or aquatic processes as indicated by poorly drained soils, hydrophytic vegetation, and various kinds of biological activity adapted to a wet environment" (Zoltai and Vitt, 1995).Occupying an area of about 390,000 km^2, the Canadian Prairie Pothole Region (PPR) is an ecologically and economically important agriculture-wetland landscape that covers almost 80% of western Canada (Wrubleski & Ross, 2011). This region's dry climate, clay-rich glacial tills, and hummocky Prairie landscape saw the formation of

numerous “potholes” (i.e., depressional wetlands, freshwater marshes) within small drainage basins that are hydrologically isolated from one another (Euliss *et al*., 1999; Conly and van der Kamp, 2001; van der Kamp and Hayashi, 2009). Depressional wetlands are filled by annual snowmelt during spring runoff as well as early season rains (van der Kamp and Hayashi, 2009). However, due to the PPR’s dynamic climate, there can be strong interannual variation between wet (i.e., abundant rainfall) and dry periods (i.e., drought); temperatures can exceed 40 °C in summer and -40 °C in winter (Conly and van der Kamp, 2001; Johnson et al., 2004). Wetlands can last for as little as two weeks in spring or up to years during a wet cycle. Regional wetland plants typically form diverse communities along moisture gradients and somewhat uniquely exhibit classic patterns of zonation by forming rough, concentric circles of vegetation (Stewart and Kantrud, 1972; Guntenspergen *et al*., 2002). PPR wetland plant communities respond to water level fluctuations in 4 phases: dry marsh (seed germination), regenerating marsh (growth of plant zonation along a hydrologic gradient), degenerating marsh (long inundation period where emergent dieback occurs) and lake marsh (permanent, open-water; Euliss *et al*., 1999). In some years, wetlands of varying permanence classes may experience the full range of inundation periods during the weather cycle (Euliss *et al*., 2004).

A disproportionately high number of species are supported by wetlands where they provide habitat and resources for waterbirds, amphibians, invertebrates, and aquatic vegetation (Bartzen *et. al*., 2010). Prairie potholes are the most productive waterfowl habitat in the world annually providing critical breeding habitat for ~21.6 million ducks (Wrubleski & Ross, 2011). This is significant as many of these migratory avian species rely heavily on an abundance of insect protein for their diet during spring staging. In general, wetlands provide numerous ecosystem services, including: water quality improvement, flood attenuation, carbon management, and biodiversity support (Zedler and Kercher, 2005). However, many wetland functions are directly or indirectly associated with macrophyte (i.e., wetland plant) production. Aquatic plants produce the annual crop of biomass and litter needed for primary production, although algae can also be a major contributor in the absence of wetland vegetation (van der Valk, 2012). Decomposition of litter is one of the most important processes in Prairie wetlands where leaching makes sugars and amino acids available for microorganisms and mineralization breaks down organic molecules (van der Valk, 2012). Because of the large amounts of litter produced in these systems, oxygen is often depleted quickly producing anaerobic environments.

High wetland productivity is sustained by the release of nutrients during dry marsh conditions that then foster new aquatic plant communities and sharply increase wetland productivity after re-flooding takes place (van der Valk, 1981; Johnson, 2004).

1.3.2 Wetland classification

The Canadian Wetland Classification System is based on categorizing important ecosystem processes such as water and carbon budget, and environmental parameters such as water quality and quantity (Zoltai and Vitt, 1995). In contrast, many previously developed Prairie wetland classification systems are based on plant community zonation and hydrological information (e.g., basin fill; Stewart and Kantrud, 1971), while others include more complex information such as capacity, drainage, and wetland configuration (Millar, 1976). One key criticism is that most widely accepted classification systems do not consider geomorphic setting and/or the temporal nature of wetlands (Euliss *et al*., 2004). In this dissertation, I used a hybrid of both Stewart and Kantrud (1971) and Millar (1976) where vegetation presence or absence and/or the distributional pattern of zones are the primary determining factors used in classifying Prairie wetlands. Vegetation zones indicate hydrologic factors such as water regime, salinity, and anthropogenic disturbance (e.g., tillage; Stewart and Kantrud, 1972; DeKeyser *et al*., 2003) and plants can be used as a surrogate for assessing changes in hydrologic function and wetland quality (Galatowitsch et al., 2000; (DeKeyser *et al*., 2003). According to Stewart and Kantrud (1971), the four most common vegetation zones in these systems include the wet-meadow (e.g., fine textured grasses, rushes), shallow marsh (e.g., grasses and sedges of intermediate height), deep marsh emergent (e.g., reeds, rushes), and shallow open water (e.g., floating or submerged aquatics). The five most common wetland classes are distinguished by the vegetation zone occurring in the central portion of the pond as well as level of water permanence: ephemeral (class I), temporary (class II), seasonal (class III), semi-permanent (class IV), and permanent (class V). However, most widely-used Prairie wetland classification systems do not accurately characterize adjacent land use patterns that may drastically alter the physical, hydrological, and biological wetland environment (Guntenspergen *et al*., 2002; Euliss *et al*., 2004; Bartzen *et. al*., 2010)

1.3.3 Wetland assessment systems

Although wetland assessment systems typically include classification, they move beyond delineation of wetland features to examine overall wetland condition. Wetland assessment systems are formed on 3 levels: 1) *remote assessments* (utilizing aerial imagery to predict wetland condition); 2) *rapid wetland assessments* (based on plant and landscape characteristics); and, 3) *intense assessments* (evaluation of wetlands based on intensive study of plant communities; Fennessy, *et al*., 2007). Intensive wetland studies often use an index of biotic integrity (IBI), place quadrats at numerous points within a vegetation community, collect vegetation specimens, and measure percent cover (DeKeyser, 2003; Hargiss *et al*., 2008). These health assessments, although effective, often unequally prioritize vegetation inventory as a measure of ecosystem function (Cole, 2002). Intensive assessments may also ignore variables that heavily influence wetland health such as physical alterations, hydrologic modification, or recording presence of plant zonations along moisture gradients which are indicative of ecosystem condition (Wilson and Bayley, 2012). Rapid assessments have been developed with success in states such as Ohio, Florida, North Dakota and Oregon (Mack, 2006; Fennessy, *et al*., 2007). For my dissertation research, I developed and validated my own rapid system by integrating several assessment and classification criteria (Stewart and Kantrud, 1971; Millar, 1976, DeKeyser, 2003, Fennessy *et al*., 2007) in order to examine numerous ecological and landscape variables together. Rapid methods require less time, are less expensive, and also require less taxonomic expertise allowing for greater sample sizes; they are seen as central to any type of monitoring or effective ecosystem management as they attempt to record stressor indicators (Fennessy *et al*., 2007). Canadian wetland studies of anthropogenic impacts are not comprehensive and few, if any, have examined the PPR at the landscape scale (Bartzen et. al., 2010). In order to better understand the distribution of chemical stressors (e.g., pesticides) in the environment, it is important to conduct locally intensive surveys across a range of scales that chemicals are used (Catallo, 1993; Johnson, 2002).

1.3.4 Prairie wetland stressors and wetland ecotoxicology

Over half of Prairie wetlands have historically been drained and the remaining intact wetlands are stressed by eutrophication, sedimentation, loss of vegetation zones, and increased tillage of marginal lands (Kantrud and Newton, 1996; Bartzen *et al*., 2010). The combined

stressors and alterations to the PPR have further compromised the overall ecosystem function of this ecologically and economically valuable agro-wetland landscape (Dahl and Watmough, 2007). Presently, Canada's PPR wetlands may be threatened by an increasing national reliance on agro-chemicals which could be degrading regional wetland water quality leading to a potential reduction in aquatic prey resources for wetland-dependent organisms. In particular, Saskatchewan is the greatest user of pesticides in Canada accounting for an estimated 36% of total sales (Brimble *et al*., 2005). Moreover, landscape simplification – or the shift toward large-scale production, mechanization, and mono-cropping – has further led to exponential growth in chemical inputs designed to improve agricultural yields (Tilman *et al*., 2001; Meehan *et al*., 2011). Prairie wetlands are especially susceptible to pesticide transport as they occupy topographic depressions that naturally accumulate pesticides from deposition, spray-drift, and surface/subsurface runoff (Goldsborough and Crumpton, 1998). In many cases, we do not currently understand how annual pesticide applications affect wetland ecosystems (Relyea and Hoverman, 2006). Cultivation of both wet meadow and shallow marsh vegetation communities can increase the potential for chemical inputs such as pesticides (Kantrud and Newton, 1996). Part of the objective of wetland ecotoxicology should be to determine management strategies to ameliorate impacts on these chemically-stressed systems with an immediate need to determine chemical fate in ecosystems and understand the functional alteration of landscapes by contaminants (Catallo, 1993). Identifying general patterns of pesticide effects in aquatic systems such as wetlands will offer better predictive ability in the field of ecotoxicology, particularly in terms of conservation (Relyea and Hoverman, 2006).

1.3.5 Pesticide use across Prairie Canada

Agricultural intensification and pesticide use has been directly associated with biodiversity loss in Canada and abroad (McLaughlin and Mineau, 1995; Gibbs et al., 2009). Actual pesticide sales data in Canada is presently confidential (although survey data on use is available through government); without published statistics or geographic information, it is unclear as to the amount or distribution of use across the Prairies for many agrochemicals (Environment Canada, 2011). Instead, sales data are often used to determine some indication of use in the province of purchase and the potential for exposure in the environment (Brimble *et al*., 2005). There is a need to better understand annual pesticide use patterns and distribution across

the Prairies in relation to measured concentrations in the aquatic environment. In Canada, the majority of the land base receiving pesticide inputs is located in the Prairie Provinces with 86, 78, and 89% of the cultivated area receiving herbicides, insecticides, and fungicides, respectively (Leeson and Beckie, 2014). Pesticide losses from croplands to wetlands are typically 1 to10% with potentially greater losses if rainfall events occur shortly after application (Fawcett *et al*., 1994; Cairns and Niederlehner, 1996). In Prairie agroecosystems, wetlands in flooded agricultural landscapes were found to contain an average of 19 herbicides and insecticides (Donald *et al*., 2005). Indeed, in surveys of pesticide distribution across the Prairies, numerous studies have detected compounds such as lindane, 2,4-D, atrazine, MCPA, chlorpyrifos in atmospheric samples (Messing *et al*., 2013, Messing *et al*., 2011) and regional waterbodies such as wetlands, lakes, and streams in 3 to 100% of samples (Donald and Syrgiannis, 1995; Rawn *et al*., 1999, Cessna and Elliott, 2004). Contamination by agro-chemicals such as herbicides and pesticides is a significant issue given that up to 24% of Saskatchewan's wetlands may surpass regulatory requirements for protection of aquatic life during storm events (Donald et.al., 1999).

1.3.6 General pesticide properties affecting fate and transport

Determining the fate and transport of pesticides in the environment is a complex process as no single factor (e.g., adsorption, water solubility) can be used to predict pesticide behavior. Instead, interactions between factors coupled with environmental conditions are necessary to accurately determine pesticide fate (Gavrilescu, 2005). Additionally, the short-term behavior and long-term fate of pesticides in surface waters are controlled by the properties of the individual pesticide: physical (e.g., solubility, temperature), chemical (e.g., structure, toxicity), and biological (e.g., microorganism presence; Goldsborough and Crumpton, 1998). Once applied to cropped areas, pesticides move into water bodies through surface runoff, erosion, leaching to groundwater, and to the atmosphere via spray drift and volatilisation (Relyea and Hoverman, 2006; Sarmah et al., 2004; Goldsborough and Crumpton, 1998).The ability of a compound to persist in the aquatic or terrestrial environment may involve a number of processes such as photolysis, volatilization, sedimentation, sorption/desorption, and biodegradation (Sarmah *et al*., 2004; Holvoet *et al*., 2007; Arias-Estévez *et al*., 2008). However, less is known about how pesticide fate is influenced by the interplay between abiotic (e.g., soil type, topography, water depth) and biotic factors (e.g., submerged/emergent macrophyte presence) at a range of spatial

scales. This is especially true of plant cover which is the most important characteristic influencing the retention performance of pesticides eventually leading to degradation via hydrolysis or photolysis (Stehle et. al., 2011).

1.3.7 Overview of neonicotinoid insecticide use and toxicity

Western nations such as Canada, the United States, France, Germany, and the United Kingdom, have steadily increased their use of a newer class of insecticides – the neonicotinoids. Beginning in December 2013, moratoriums were placed on use in EU member states (e.g., Italy, Germany, and France) and to a lesser extent on oilseed rape (canola) production in the United Kingdom. Highly valued for their versatility in application (e.g., foliar sprays, soil drenches; Goulson, 2013), most are used as seed treatments on major agricultural crops, including: canola, cereals (e.g., barley, wheat), corn, cotton, potatoes, and soybeans (Elbert *et al.*, 2008). Most pesticide use data in Canada and the United States remains confidential (Main *et al.*, 2014; Douglas and Tooker, 2015) with the distribution and actual scale of neonicotinoid use unknown. Neonicotinoid seed treatments are designed as a systemic that protects the roots and shoots of young plants throughout their growth while lessening the amount of insecticide used in spray or soil drenching applications (Elbert *et al.*, 2008; Jeschke *et al.*, 2010). Acting on the central nervous system, active ingredients are agonists of the post-synaptic nicotinic acetylcholine receptors (*n*AChRs) causing receptor blockage, paralysis and death at higher concentrations to insect pests (Tomizawa and Casida, 2005; Jeschke and Nauen, 2008; Goulson, 2013), but are equally effective against non-target organisms such as bees and aquatic invertebrates. There is a growing body of knowledge concerned specifically with the toxicity of neonicotinoids toward pollinators and other non-target aquatic insects (Alexander *et al.*, 2008; Krupke *et al.*, 2012; Van Dijk *et al.*, 2013; Fischer *et al.*, 2014). Although theoretically lessening the impact on the environment, >90% of the active ingredient may enter surrounding soils (Goulson, 2013) where patterns of persistence can vary from a few days to years (DeCant and Barrett, 2010; Goulson, 2013). Neonicotinoids are also highly water soluble, yet the specific fate of this insecticide is poorly understood (Goulson, 2013; Anderson *et al.*, 2015).

1.3.8 Neonicotinoid physico-chemical properties and environmental fate

The physico-chemical properties of neonicotinoids – specifically their high water solubility, low soil adsorption (log K_{OC}), and partitioning properties (low log K_{OW}) – readily promote transport of these insecticides into aquatic systems through surface and subsurface runoff (Beketov and Liess, 2008; Morrissey *et al.*, 2015; Table 1.1).

Table 1.1 Chemical properties and environmental persistence of four neonicotinoid insecticides measured in this study: acetamiprid, clothianidin, imidacloprid, and thiamethoxam.

Compound [ab]	Water Solubility (mg/L) @ 20 °C	Lipophilicity (log K_{OW})	Soil Affinity (log K_{OC})	Vapor Pressure (mm Hg) @ 20 °C	Soil Persistence (DT_{50} - Days)	Water Photolysis (DT_{50} - Days)	Water Hydrolysis (DT_{50} - Days)[c]
Acetamiprid	2950	0.80	2.3	4.4×10^{-5}	2-20	34.0	Stable: 420
Clothianidin	340	0.91	2.08	9.8×10^{-10}	13-1386	<1	Stable: 14.4
Imidacloprid	610	0.57	2.19-2.90	3.0×10^{-12}	104-228	<1	Stable: >1 yr
Thiamethoxam	4100	-0.13	1.75	4.95×10^{-11}	7-72	2.7-39.5	Stable: 11.5

[a] Table adapted from information from Morrissey *et al*.(2015) and Hazardous Substances Data Bank (HSDB).

[b] CAS Numbers for each active ingredient are as follows: acetamiprid (135410-20-7), clothianidin (210880-92-5), imidacloprid (138261-41-3), and thiamethoxam (153719-23-4)

[c] Compounds are stable to hydrolysis under acidic or neutral conditions.

Neonicotinoids bind tightly to dry soils (Gupta *et al*., 2008) and can become persistent for long periods (e.g. clothianidin DT_{50} = 1386 d in North Dakota; PMRA, 2004) where they are expected to persist in colder regions due to both lower temperatures and sunlight intensity (Bonmatin *et al.*, 2014). Active ingredients also accumulate in soils over time (Bonmatin *et al.*, 2005; Jones *et al.*, 2014), but aged soil residues, although tightly bound, may still move with particulates in solution (Cox *et al.*, 1998). Persistence in soils influences the likelihoood that these insecticides can be transported into regional waters. However, there is a paucity of data on how these insecticides move into wetlands during different seasons and their ultimate fate in the wetland environment. Soil persistence is largely dependent on factors such as application rate, pH, and temperature whereas in regional waterbodies high turbidity, acidity, water depth, and other biological shading (e.g, algae cover) will likely increase chemical persistence (Guzsvany *et al*., 2006; Morrissey et al., 2015). To my knowledge, no studies have examined the numerous abiotic and biotic wetland variables that may affect neonicotinoid fate in wetlands. Neonicotinoids typically exhibit peak concentrations in water within 24 h post-application and breakdown following first-order kinetics (i.e., rapid loss over the first few days with a slower second phase; Armbrust and Peeler, 2002). However, it is unclear if they are persisting in wetlands and how concentrations may change in these aquatic environments over time. The same can be mentioned as to the extent that margin vegetation or other plants may affect fate and/or draw up neonicotinoids from arable soils (Goulson, 2013).

1.3.9 Conclusion

Interdisciplinary approaches to wetland ecotoxicological studies need to be developed in order to address landscape-level understanding of pesticide distribution, fate, and effects of agrochemicals on waterbodies (Catallo, 1993). This is especially true of complex ecosystems such as Prairie wetlands. In principle, the integration of wetland assessment and classification criteria provides a framework for rapidly analyzing numerous ecological variables (e.g., biological, physical, and hydrological) across a range of scales (Stewart and Kantrud, 1971; Fennessy *et al*., 2007: Kotze *et al*., 2012). However, it is important to note that most assessment systems do not include chemical stressors as part of their criteria (Kotze *et al*., 2012). Although neonicotinoid toxicity toward bees and other non-target species has been well-documented (Krupke *et al*., 2012; Van Dijk *et al*., 2013), we lack extensive biomonitoring of aquatic systems

in agriculturally-intensive regions such as the Canadian Prairies (Goulson, 2013; Anderson *et al*., 2015). The lack of extensive data on natural pesticide concentrations further presents a hurdle in evaluating the valididty of concentrations used in experiments (Relyea and Hoverman, 2006). Further, there is a clear knowledge gap in identifying features that drive neonicotinoid fate and transport in natural agroecosystems and how biological factors such as vegetation mitigate or uptake these insecticides from surrounding soils and/or waterbodies (Goulson, 2013; Anderson *et al*., 2015; Botías *et al*., 2015).

1.4 Thesis Structure

This thesis is presented in the dissertation by manuscript style and follows the guidelines set out by the University of Saskatchewan's College of Graduate Studies and Research. It has been structured into four main body manuscripts (i.e., chapters) that synthesize data on neonicotinoid use across Prairie Canada in tandem with wetland ecology/sampling from four years of field studies and statistical analyses.

Chapter 2 examines the estimated frequency of use of neonicotinoid seed treatments across Prairie Canada in relation to a regional wetland survey identifying the number of detections and concentration levels found in wetland water. Using GIS, I modeled the spatial distribution of neonicotinoid use in the agriculturally dominated regions of southern Manitoba, Saskatchewan, and Alberta by combining remotely-sensed crop maps with confidential sales data and standard pesticide application rate recommendations. I used these maps to identify areas of high frequency of neonicotinoid use in relation to high wetland density. I then sampled a subset of typical agricultural wetlands (both water and sediment) to evaluate current levels of neonicotinoids found in the environment and potential changes in concentration over the agricultural growing season (spring 2012 to spring 2013).

In Chapter 3, I used a subset of agricultural wetlands (8 in previously treated fields, 8 in untreated fields) to identify the major pathways (e.g., top- and bottom-layer snow, meltwater, soil particulate) of neonicotinoid contamination to wetland water in spring. Additionally, I followed the same wetlands over time to assess neonicotinoid concentration changes across different wetland classes from ice-off to pre-seeding. The major goal of this chapter was to ascertain why spring wetland waters contain measureable neonicotinoid concentrations before annual insecticide applications have begun.

In Chapter 4, I identify the major drivers of both neonicotinoid detection and concentration in 238 Prairie wetlands found across central Saskatchewan. Through explorative analysis and modeling of 59 different ecological, hydrological and landscape variables, I was able to predict those factors which most influenced neonicotinoid detections and concentrations across a range of wetland classes.

Chapter 5 examines the ability of common wetland macrophytes to accumulate and/or mitigate neonicotinoid contamination from entering Prairie wetlands. A field survey of 16 different wetland plant species collected from 10 vegetated wetlands in clothianidin-treated canola revealed that a number of common species (e.g., *Equisetum arvense*) had detectable levels of neonicotinoids within their tissues. This information was compared to a thiamethoxam-dosed microcosm study of two species, *Alisma triviale* and *Typha latifolia*, to assess the relative ability of wetland plants to uptake neonicotinoid active ingredients.

My final chapter is a synthesis of major findings (and limitations) from the preceding four data chapters, as well as general and specific conclusions about neonicotinoid distribution and fate in the wetland environment. I place my findings in context of greater research importance and contributions to studies of Prairie wetlands, pesticides, and agroecosystems. Then, I briefly present the implications for wetland sustainability in addition to prospects for future research directions based on a number of questions that arose from my dissertation.

1.5 Authorship

This dissertation research is a compilation of four years of fieldwork and analyses primarily conducted by the author (AM). Each main chapter is written in a manuscript style and either has been published or will be submitted to selected journals in the fields of ecotoxicology, agroecosystems, and/or wetlands. My supervisor, Dr. Christy Morrissey, co-authored chapters 2, 3 and 4 in addition to co-authorship or involvement by Drs. Nicole Michel, John Headley, Allan Cessna and Mr. Kerry Peru. However, as primary author, I designed the studies, conducted all field research (including sample collection and preparation), analyzed my data, and wrote the thesis. My supervisor provided research funding and advice on study design, aided in interpretation of results and overall content, and reviewed and edited drafts of each of the manuscripts written by the primary author. Additionally, all water, sediment, and plant tissue analyses were completed by JH, AC and KM at Environment Canada. Statistical mentoring and code development for advanced modeling techniques was done in collaboration with NM. Other

individuals (not listed here) also assisted with field data collection and are subsequently acknowledged at the end of each chapter.

PREFACE TO CHAPTER 2

Neonicotinoid seed treatments currently dominate the insecticide market and are used extensively on Canada's major Prairie crops including canola, cereals, corn, legumes, and soybeans. However, actual pesticide sales data in Canada remains confidential and the distribution of neonicotinoid use, incidence, and level of contamination remains unreported. It is unclear as to how these insecticides may affect wetlands surrounded by agricultural production. Further, tools such as geospatial mapping combined with temporal assessments of wetland water and sediment may highlight areas of greatest concern for Prairie wetland conservation. The objective of this chapter was to: 1) develop geospatial maps of estimated current neonicotinoid use across the Prairie Provinces in relation to annual crop rotations; and, 2) survey levels of neonicotinoids in both water and sediment in a subset of study wetlands surrounded by a range of agricultural crops over one full year.

Chapter 2 is published in PLOS ONE*. See: Main, A.R., Headley, J.V., Peru, K.M., Michel, N.L., Cessna, A.J. and Morrissey, C.A. (2014). Widespread Use and Frequent Detection of Neonicotinoid Insecticides in Wetlands of Canada's Prairie Pothole Region. *PLOS ONE* 9 (3): e92821. DOI: 10.1371/journal.pone.0092821.

*Minimal changes have been made to the original published manuscript text including clarification of the methods and reference formatting.

CHAPTER 2: WIDESPREAD USE AND FREQUENT DETECTION OF NEONICOTINOID INSECTICIDES IN WETLANDS OF CANADA'S PRAIRIE POTHOLE REGION

2.1 Abstract

Neonicotinoids currently dominate the insecticide market as seed treatments on Canada's major Prairie crops (e.g. canola). The potential impact to ecologically significant wetlands in this dominantly agro-environment has largely been overlooked while the distribution of use, incidence and level of contamination remains unreported. We modelled the spatial distribution of neonicotinoid use across the three Prairie Provinces in combination with temporal assessments of water and sediment concentrations in wetlands to measure four active ingredients (clothianidin, thiamethoxam, imidacloprid and acetamiprid). From 2009 to 2012, neonicotinoid use was increasing; by 2012, applications covered an estimated ~11 million hectares (44 % of Prairie cropland) with >216,000 kg of active ingredients. Thiamethoxam, followed by clothianidin, were the dominant seed treatments by mass and area. Areas of high neonicotinoid use were identified as high density canola or soybean production. Water sampled four times from136 wetlands (spring, summer, fall 2012 and spring 2013) across four rural municipalities in Saskatchewan similarly revealed clothianidin and thiamethoxam in the majority of samples. In spring 2012 prior to seeding, 36% of wetlands contained at least one neonicotinoid. Detections increased to 62% in summer 2012, declined to 16% in fall, and increased to 91% the following spring 2013 after ice-off. Peak concentrations were recorded during summer 2012 for both thiamethoxam (range: <LOQ - 1490 ng/L, canola) and clothianidin (range: <LOQ – 3110 ng/L, canola). Sediment samples collected during the same period rarely (6%) contained low neonicotinoid concentrations (which did not exceed 20 µg/kg). Wetlands situated in barley, canola and oat fields consistently contained higher mean concentrations of neonicotinoids than in grasslands, but no individual crop singularly influenced overall detections or concentrations. Distribution maps indicate neonicotinoid use is increasing and becoming more widespread with concerns for environmental loading, while frequently detected neonicotinoid concentrations in Prairie wetlands suggest high persistence and transport into wetlands.

2.2 Introduction

Degradation of aquatic ecosystems from chemical inputs is a global concern because of the loss of ecosystem services provided through water supplies, food resources and habitat for species of fish and wildlife. Wetlands are some of the most sensitive, biologically diverse, and globally productive ecosystems (Erwin, 2009). Worldwide, the rate of loss and deterioration of wetlands is accelerating due to increasing anthropogenic impacts affecting their overall ecological condition (Bedford *et al.*, 2001). Wetlands in agricultural areas in Canada are under serious threat from expanding agricultural intensification; specifically, increased reliance on chemical fertilizers and pesticides (herbicides, fungicides, and insecticides). There is a growing concern that these inputs are degrading wetland water quality and, consequently, impacting aquatic and wetland-dependent terrestrial species. With over 50% of the wetlands in the Prairie Pothole Region (PPR) of Canada historically drained, the remaining intact wetlands are under stress due to eutrophication, sedimentation, loss of vegetation and tillage of marginal lands as a result of agricultural activity (Bartzen *et al*., 2010). Farming has shifted toward large-scale production, mechanization and mono-cropping. Researchers estimate an exponential growth in chemical inputs designed for improved agricultural yields – specifically, the increased use of insecticides (Meehan *et al*., 2011).

Current agricultural practices are dependent on a newer class of insecticides, the neonicotinoids. Valued for their versatility in application (Elbert *et al*., 2008; Jeschke *et al*., 2010; Blacquière *et al*., 2012) and widely used throughout Europe and North America, these chemicals represent the fastest growing class of insecticides globally since the introduction of the pyrethroids. The extensive use of the neonicotinoids is largely due to their effectiveness and broad spectrum toxicity to a wide range of pests (Jeschke and Nauen, 2008). Eighty percent of all treated seeds are coated with a neonicotinoid insecticide (Jeschke *et al*., 2010). Seeds of the major Prairie crops in Canada (e.g., canola, wheat, barley, oat and field pea) are commonly coated with one of the neonicotinoid active ingredients clothianidin, imidacloprid, or thiamethoxam while acetamiprid is also used on fruit or leafy vegetable crops. The Canadian Prairie Pothole Region (PPR) consists of 39 million hectares (ha) and accounts for 98% of the country's canola production – over 8.5 million ha were seeded in 2012 (Statistics Canada, 2012) of which nearly all were seeded with neonicotinoid-treated seed (PMRA pers. comm).

Neonicotinoids - systemic insecticides - contain an active ingredient that translocates throughout the growing plant and acts on the nervous system of insect pests (Tomizawa and Casida, 2005). Recent concern over this class of insecticides is, in part, due to their acute toxicity to non-target insects such as bees and aquatic invertebrates (Alexander *et al*., 2007; Stoughton *et al*., 2008; Girolami *et al*., 2009; Pestana *et al*., 2009; Krupke *et al*., 2012; Whitehorn *et al*., 2012). In addition, some of the neonicotinoids have relatively long half-lives in soil (e.g., thiamethoxam DT_{50} = avg. 229 days; clothianidin DT_{50}= 148 – 1,155 days) and high water solubility (e.g., thiamethoxam = 4,100 mg/L; clothianidin = 327 mg/L; HSDB, 2012) leading to environmental persistence and high potential for transport into surface waters via surface runoff or groundwater discharge (Starner and Goh, 2012; Van Dijk *et al*., 2013).

From 1971 to 1991, pesticide use in Canada increased by 500% resulting in a greater quantity of pesticides susceptible to transport (Goldsborough and Crumpton, 1998). Today, more pesticides are used in the Prairies than any other region of Canada (Brimble *et al*., 2005). Wetlands in the PPR typically occupy topographic depressions that naturally accumulate surface runoff which may contain pesticides from adjacent/surrounding agricultural fields (Goldsborough and Crumpton, 1998). Millions of PPR wetlands drain surrounding agricultural fields and accumulate snowmelt and (to a lesser extent) summer rainfall (Conly and van der Kamp, 2001; van der Kamp and Hayashi, 2009) potentially making them susceptible to neonicotinoid contamination. For example, up to 24% of Saskatchewan's wetlands may surpass pesticide regulatory requirements for protection of aquatic life during storm events (Donald *et al*., 1999). During high rainfall events, Prairie wetlands in flooded agricultural landscapes were found to contain an average of 19 herbicides and insecticides (Donald *et al*., 2005).

Many western nations are examining the distribution and use of neonicotinoids along with impacts on ecosystem health (Van Dijk *et al*., 2013). However, the actual distribution and concentration of neonicotinoids in North American surface water systems remains poorly known with the exception of limited published studies focused on imidacloprid in rivers and streams (Phillips and Bode, 2004; Starner and Goh, 2012; Xing *et al*., 2013) and one study reporting thiamethoxam and acetamiprid detections in playa wetlands of Texas (Anderson *et al*., 2013). In the PPR agricultural-wetland landscape, the actual distribution of use of neonicotinoids and their levels in agricultural wetlands remains unknown. Therefore, our objectives were to: 1) develop

geospatial maps of current neonicotinoid use within the PPR in relation to annual crop plantations and 2) survey levels of neonicotinoids in water and sediment of a subset of wetlands surrounded by different crops (grasslands, barley, canola, oat, wheat and field pea) through time. We hypothesized that neonicotinoid applications would be highest in areas of intensive canola production and neonicotinoid concentrations and detections in wetlands would similarly be higher in canola fields, particularly during the summer growing season.

2.3 Methods

2.3.1 Study Area for Wetland Sampling

Our study was carried out across a 32-km^2 area in central-east Saskatchewan. Water and sediment samples were collected from wetlands situated in agricultural fields near the communities of St. Denis (52º 10'22" N, 106º 5'57" W), Colonsay (51º 59' 0" N, 105º 53' 0" W), Lanigan (51º 51' 0" N, 105º 2' 0" W) and Humboldt (52º 12' 7" N, 105º 7' 23" W). The study fields were selected to represent the range of Prairie crop types located in zones of intensive agricultural production and neonicotinoid use as well as a high density of pothole wetlands.

2.3.2 GIS Mapping of Neonicotinoid Applications

Pesticide sales reporting in Canada is currently considered confidential and use of seed treatments for specific crops are poorly monitored. To estimate the spatial distribution of neonicotinoid use across the Canadian Prairies, we integrated standard pesticide application rate recommendations for registered uses of seed treatment products and their associated crops (Government of Saskatchewan, 2011), percentage of each crop treated with neonicotinoids (2009-2010 confidential PMRA data), and remote-sensing field-level crop inventory maps (Agriculture and Agri-Food Canada) into a geographic information system (GIS; ArcMap 10, Environmental Systems Resource Institute, Redlands, CA). Data on PPR cropland distribution was derived from Agriculture Canada's remotely sensed land cover maps at 56-m resolution (2009-2010) and 30-m resolution (2011-2012). For our analysis, cropland of interest included all land potentially planted with treated seed including: barley, canola, corn, dry bean, field pea, mustard, oat, soybean and wheat. Percentages of singular treated crops were then extracted from remote sensing crop maps based on available 2010 confidential PMRA data to isolate treated

land areas from total planted areas. Integrated maps were individually created for three neonicotinoid compounds (thiamethoxam, clothianidin, imidacloprid) and year (2009-2012). Because we were primarily interested in seed treatments of grain crops, acetamiprid maps were not compiled because Prairie crop-use data were limited to potato which is treated both with a seed treatment and foliar spray. We determined the neonicotinoid application rate via treated seed by multiplying grams of neonicotinoid active ingredient (AI) per kilogram of seed by the seeding rate of kilograms seed per hectare. This produced a rate of grams of active ingredient per hectare (g AI/ha). For crops potentially using more than one application rate, we conservatively used median recommended guidelines (e.g., thiamethoxam: barley = 13 g/ha, canola = 21 g/ha, beans = 26 g/ha). We calculated the pixel equivalent of a hectare for all raster maps by dividing raster resolution by size of an actual hectare (e.g., resolution = 56 m x 56 m / ha = 100 m x 100 m). We then used a conditional statement in ArcMap Spatial Analyst tools to multiply the hectare equivalent by calculated application rate (g AI per specific crop) to determine an estimated value for each hectare planted to one crop type. Because the majority of field crops are planted on a quarter section level (65 ha), all individual crop maps by AI were merged together (by specific year) and summed to estimate total neonicotinoid distribution throughout the PPR.

2.3.3 Water Sampling

We used the Dominion Land Survey system (ISC, 2013) which divides agricultural land across the Canadian Prairies into 1.6-km^2 sections (260 ha) containing four quarter sections (65 ha) to delineate zones for wetland sampling because crops are planted at the quarter section scale. We sampled, where available, three replicate wetlands from each of 50 quarter sections across a range of wetland classes (Class II: temporary ponds; Class III: seasonal ponds; Class IV: semi-permanent ponds; and Class V: permanent ponds). Fields were randomly selected to represent five agricultural crop types in the study area (canola, barley, wheat, oat and field pea) in addition to grasslands/hayfields. In total, water samples from 136 wetlands in 50 quarter sections were collected for analysis; 89% of wetlands sampled were situated in crop fields as follows: canola (40%), barley (20%), wheat (18%), oat (11%), field pea (0%) with 11% of the wetlands situated in grassland and hayfield (reference) areas. In spring 2012, there were no wetlands situated in fields seeded to field pea the previous year, but wetlands in pea fields were

sampled in subsequent water collections. Water samples from the same wetlands were collected four times: between snowmelt and seeding in spring 2012 (April), during the growing season in summer 2012 (June), after harvest in fall 2012 (September) and again between snowmelt and seeding in spring 2013 (May). Collection sites were accessible by foot and samples were collected centrally in each wetland beyond surrounding edge vegetation and, where possible, distant from submerged aquatic vegetation. One litre (L) of water was collected using a subsurface grab at a depth of 10 cm in chemically cleaned (acetone: hexane washed) amber glass jars. Bottles were sealed with Teflon-lined caps and then stored in the dark during transport and refrigerated at 4ºC until analysis.

The type of crop surrounding each wetland was determined from landowner crop rotation schedules or by plant identification. GPS coordinates and photographs of each study wetland were recorded to ensure the same wetlands were sampled in subsequent sampling periods.

2.3.4 Sediment Sampling

During the summer 2012 water collection, we also collected sediment cores from each study wetland. Sediment sampling involved collection of one core 0- to 6-cm depth sample from each of three wetland zones (one core per zone): centrally, the zone of emergent vegetation and that of submerged vegetation. Sediment was collected using a 1.2-m black PVC pipe with a 15-cm diameter opening and 0.6-cm holes drilled into the lower sides of the pipe to allow water to evacuate during coring. The combined sediment cores were pooled to yield a sediment sample of approximately 1 kg. Sediment was placed in polyethylene freezer bags, transported to the laboratory in a large cooler and then immediately placed in a freezer at -20ºC until analysis.

2.3.5 Chemical Analysis

Wetland water and sediment samples were analyzed at the National Hydrology Research Centre, Environment Canada, Saskatoon, SK using methods adapted from that of Xie *et al*. 2011. Analytical standards of thiamethoxam, clothianidin, imidacloprid and acetamiprid were from Chem Service (West Chester, PA, USA) and the internal standard, d_4-imidacloprid, from CDN Isotopes (Pointe-Claire, Quebec, CA).

Sample Extraction: In brief, water samples (500 mL) were passed through Oasis HLB cartridges (Waters, Mississauga, Canada) which had been sequentially conditioned with methanol (10 mL) and water (10 mL). After sample loading, the cartridges were washed with de-ionized water (5 mL) to remove salts and the cartridges were dried under vacuum for 5 min. The retained analytes were eluted with methanol (10 mL), the eluates were evaporated to dryness and the extract residues reconstituted in 500 µL of water followed by addition of the internal standard. Sediment samples (5.0 g wet-weight) and acetonitrile (10 mL) were sonicated (30 min) and then centrifuged (15 min @ 5000 rpm) and the supernatant decanted. Following a second sonication and centrifugation, the combined decantates were evaporated to ~ 1 mL, taken to a final volume of 2 mL with water, and internal standard added.

LC/MS/MS Analysis: A Waters 2695 Alliance HPLC system (Waters Corp., Milford, MA), consisting of a solvent degassing unit, pump and autosampler, was used with a Waters XTerra MS-C_8 (3.5-µm dia. particle size) column (2.1- x 100-mm) (Waters Corp., Milford, MA) at 30ºC. Isocratic elution of the analytes was achieved with an 80/20 mix of solvent A (100% water and 0.1% formic acid) and solvent B (90% acetonitrile, 10% water and 0.1% formic acid). The run time was 10 min and the injection volume was 20 µL.

The neonicotinoid insecticides were quantitated (internal standard method) and their presence confirmed using the Micromass Quattro Premier triple quadrupole mass spectrometer (Waters Corp., Milford, MA) equipped with an electrospray ionization interface set to positive ion mode. Ionization and MS/MS conditions were optimized by infusing a 0.5 mg/L solution of each insecticide into the ion source in a 50:50 (v/v) acetonitrile:water solution with a syringe pump. MRM transitions, selected from the product ion scan and optimal cone voltages and collision energies for each neonicotinoid are provided in Table S1. Other instrumental settings were as follows: source temperature, 90ºC; capillary voltage, 3.00 kV; extractor voltage, 5.00 V; desolvation temperature, 240ºC; nitrogen desolvation gas flow rate, 476 L/ h; nitrogen cone gas flow rate, 38 L/h; nitrogen nebulizer gas flow rate was at maximum flow; multiplier voltage, 657 V; and the interchannel delay was 0.10 s. Argon was used as the collision gas at a pressure which increased the Pirani gauge reading to 3.12×10^{-4} mbar. Resolution was set to achieve unit mass resolution for quadrupole 1 and approximately 2 amu resolution for quadrupole 3.

A four-level calibration curve (5 to 50 µg) was analyzed before and after each batch of 10 samples which also contained a laboratory or field blank and a fortified sample. Limits of quantification (LOQ) in water were as follows: thiamethoxam, 1.8 ng/L; clothianidin, 1.2 ng/L; imidacloprid 1.1 ng/L; and acetamiprid, 0.5 ng/L. Mean recoveries (n = 33) from Milli Q® (n = 8) and river (n = 3) water each fortified at 500, 100 and 50 ng/L were as follows: thiamethoxam, 88.8 ± 3.4%; clothianidin, 78.9 ± 5.4% (mean ± SD); imidacloprid, 85.9 ± 3.9% and acetamiprid, 89.6 ± 3.7%. Mean recoveries from sediment fortified at 20 µg/kg (n = 5) were as follows: thiamethoxam: 73.6 ± 5.2%; clothianidin: 72.3 ± 7.0%; imidacloprid: 73.5 ± 7.1%; and acetamiprid: 74.5 ± 5.9%. Limits of quantification (LOQ) in sediment were as follows: thiamethoxam, 3.0 µg/kg; clothianidin, 1.3 µg/kg; imidacloprid 1.4 µg/kg; and acetamiprid, 0.9 µg/kg. All neonicotinoid concentrations were corrected for recovery and all laboratory and field blanks were below detection.

2.3.6 Statistical Analysis

Given the structural similarity of clothianidin, thiamethoxam, imidacloprid and acetamiprid and their cumulative and irreversible binding to insect nicotinic acetylcholine receptors, individual neonicotinoids are assumed to be additive in relative toxicity (Sanchez-Bayo, 2013). Therefore, concentrations of multiple neonicotinoids detected in a given sample were summed on a concentration basis and presented as total neonicotinoids.

We used a general linear mixed model (GLMM) in package lme4 in R (R Core Team 2013) to investigate the effects of crop type (grassland, barley, canola, field pea, oat, wheat) on changes in wetland total neonicotinoid concentration over one full agricultural growing season (April 2012 to May 2013). A GLMM with a Gaussian distribution was used because total neonicotinoid concentration met the assumption of normally-distributed residuals after log transformation. Crop type and time were fixed effects; wetlands nested within quarter sections and the slope of change in neonicotinoid concentrations over time were random effects; and baseline (spring 2012) neonicotinoid concentration and prior year's (2011) crop type were covariates. We had an unbalanced design because some ponds could not be resampled due to wetland drawdown (fall 2012) and spring 2013 overflooding. We used Akaike's information criteria (AIC) to identify the best distribution and to decide whether to retain slope and intercept random effects (Akaike, 1987). Significant interactions of crop type and time were examined

using *post-hoc* testing of interaction contrasts in package "phia" (Rosario-Martinez, 2013). We corrected for multiple comparisons and associated Type I errors using the Dunn-Šidák correction, because it has more power than Bonferroni (Gotelli and Ellison, 2004).

2.4 Results

2.4.1 Neonicotinoid distribution in the Prairies

Our predictive maps indicated broad neonicotinoid distribution and application rates across the Canadian Prairies (range: >0 - 70 g/ha) (Fig. 2.1) with further GIS analysis showing a trend of increasing use over time.

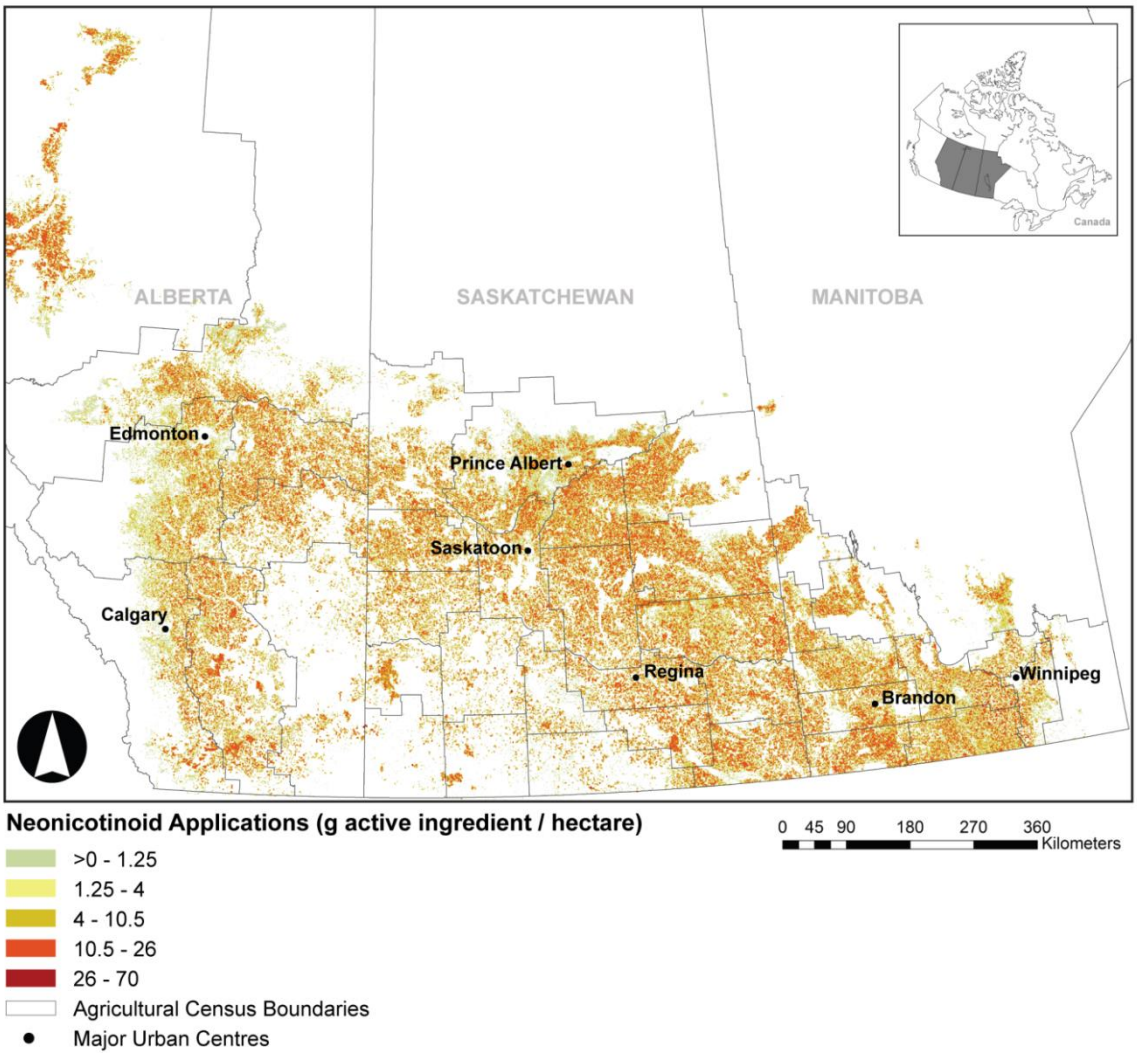

Figure 2.1 Map of modelled distribution of neonicotinoid use across Prairie Canada: Alberta, Saskatchewan and Manitoba (2012). Neonicotinoid application rates (g AI/ha) represent the sum total of clothianidin, imidacloprid and thiamethoxam across an agricultural quarter section (65-ha field) on all crops predicted to be treated with neonicotinoid seed treatments. Acetamiprid is not included because Prairie crop-use data were limited to potato which is treated both with a seed treatment and foliar spray. g AI/ha = grams of active ingredient per hectare.

By 2012, nearly 11 million hectares (est. total Prairie cropland = 25 million ha) of cropland across the Canadian Prairies was estimated to be treated with clothianidin, thiamethoxam and imidacloprid; an approximate 30% increase from 2009 (7.7 million ha; Table 2.1, Fig. 2.2). Most treated areas fell in the medium range of application rates (4 – 10.5 g/ha). We conservatively estimate that total combined mass of neonicotinoids used across Alberta, Saskatchewan and Manitoba ranged between 129,000 kg (2010) to 216,000 kg (2012; Fig. 2.2). This also represents a significant proportion of the total annually seeded cropland in the Prairies ranging from 31% in 2009 to 44% in 2012 (Table 2.1). Remote sensing data of cropland in Manitoba was not completed by Agriculture Canada in 2010, and therefore not included, which may explain the decrease in estimated neonicotinoid use. The increasing trend is evident in spite of the wet springs of 2010 and 2011, when a substantial area (2.9 million ha in 2010 and 3.1 million ha in 2011) of cropland was not seeded (Table 2.1).

Table 2.1 Estimated distribution of cropland area treated with neonicotinoids in the Prairie Pothole Region (PPR) of Canada (2009-2012). Area and percentage of seed treatment applications are separated into 5 categories defined by application rates. g AI/ha = grams of active ingredient per hectare. Bolded values are the total area treated and percentage of Prairie croplands[1].

Year[2]	Application Category	Application Rate (g AI/ha)	% Within Category	Est. Area Treated (millions ha)
	Low	>0 - 1.25	23.2	2.55
	Low-Medium	1.25 - 4	20.6	2.27
2012	Medium	4 - 10.5	30.2	3.32
	Medium-High	10.5 - 26	25.8	2.84
	High	26 - 70	< 1	0.013
				10.9 (44 %)
	Low	>0 - 1.25	21.9	2.05
	Low-Medium	1.25 - 4	22.7	2.12
2011	Medium	4 - 10.5	31.6	2.96
	Medium-High	10.5 - 26	23.8	2.23
	High	26 - 70	< 1	0.006
				9.37 (42 %)
	Low	>0 - 1.25	15.8	1.05
	Low-Medium	1.25 - 4	23.5	1.55
2010	Medium	4 - 10.5	37.1	2.45
	Medium-High	10.5 - 26	23.5	1.55
	High	26 - 70	< 1	0.007
				6.61 (30 %)
	Low	>0 - 1.25	17.5	1.36
	Low-Medium	1.25 - 4	24.6	1.90
2009	Medium	4 - 10.5	37.0	2.87
	Medium-High	10.5 - 26	20.7	1.61
	High	26 - 70	< 1	0.008
				7.75 (31 %)

[1] Total PPR cropland in production based on Statistics Canada Field Crop Reporting Series: *July 2009, July 2010, July 2011, July 2012: Estimates of Principal Field Crops.*

[2] In both 2010 (Est. 2.9 million ha) and 2011 (Est. 3.1 million ha), wet spring conditions increased the amount of cropland that went unseeded.

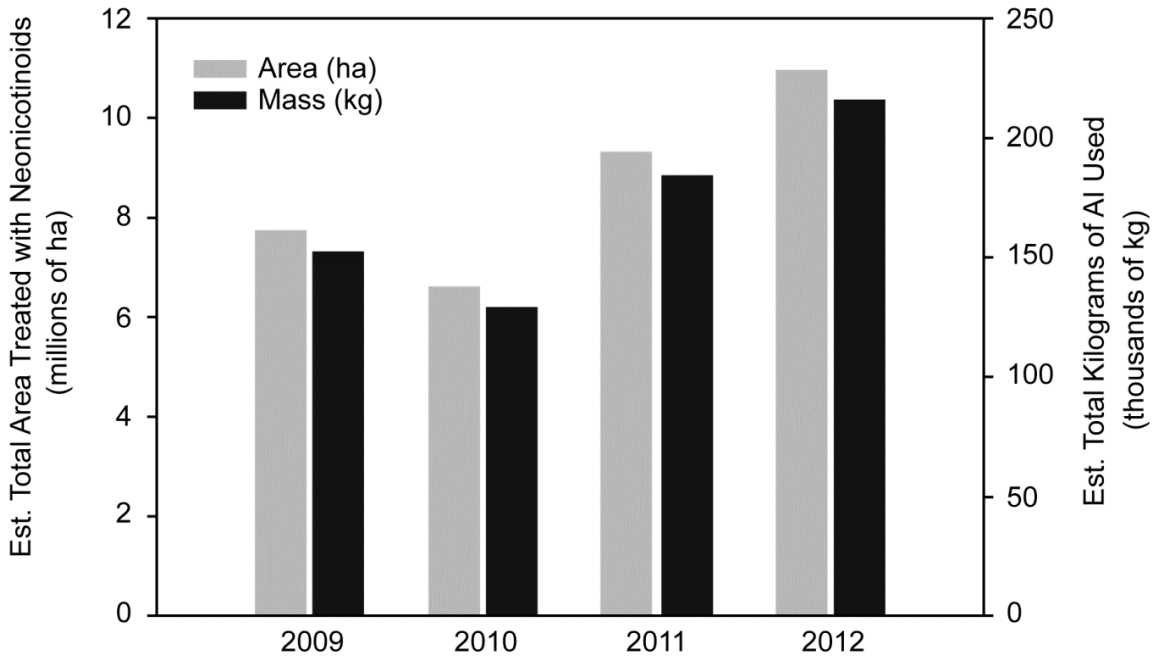

Figure 2.2 Estimated total neonicotinoid distribution across Prairie Canada. Area of total agricultural land (millions of ha) using a neonicotinoid seed treatment and estimated total mass (kg) of active ingredient (AI) applied across the Canadian Prairie region from 2009 to 2012. Composite area and mass values include all predicted treated crop types and neonicotinoid active ingredients (clothianidin, imidacloprid and thiamethoxam) based on extrapolation of mapped distribution.

Neonicotinoid treated areas and application rates (g/ha) differ by crop and active ingredient. In 2009, the dominant crops (by area) with neonicotinoid seed treatments ranked as follows: canola > wheat > corn > field pea > barley > oat. By 2012, that ranking had changed slightly to canola > wheat > soybean > corn > barley > field pea > dry bean > oat. Although canola and wheat seed treatments cover the largest area, field pea treated with thiamethoxam was calculated to have the highest application rate (70 g/ha) while oat had the lowest calculated application rate (12 g/ha). Thiamethoxam (5.8 million ha) covered the broadest spatial extent due to the range of crops on which it is currently used as a seed treatment (e.g., canola, wheat, barley). Clothianidin (5.1 million ha) was the second most widely used neonicotinoid whereas imidacloprid (45,000 ha) was substantially less. The application area for acetamiprid was not calculated because Prairie crop-use data were limited to potato which is treated both with a seed treatment and foliar spray. Overall, maximum neonicotinoid use occurred in regions with

intensive canola (Peace River region of Alberta, central Saskatchewan and southwestern Manitoba) and soybean production (southeastern Manitoba). Our results suggest that the neonicotinoids are widely used in the Canadian Prairies and that PPR wetlands are generally surrounded by crops treated with neonicotinoids which likely increases their risk of contamination with neonicotinoid insecticides.

2.4.2 Neonicotinoid concentrations in water

In spring 2012, between snowmelt and seeding, 36 % of (49/136) wetlands sampled contained at least one neonicotinoid. By summer 2012, the number of wetlands with detectable concentrations of neonicotinoids had increased to 62 % (83/134) after seeding (Table 2.2). After harvest (fall 2012), 16 % (13/80) wetlands contained trace neonicotinoid concentrations. Of the wetlands that were accessible for re-sampling the following spring (2013), 91% (82/90) had detectable neonicotinoid concentrations. At the field level, neonicotinoids were detected in wetlands on 29 of 52 quarter sections in spring 2012 (56 %); 37 of 49 quarter sections in summer 2012 (76 %); 11 of 38 quarter sections in fall 2012 (29 %) and 33 of 35 quarter sections in spring 2013 (94 %). Detections of neonicotinoids in wetlands included all crop types and grassland samples.

Table 2.2 Summary of detections, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients in water from Prairie wetlands of central Saskatchewan (2012-2013). Concentrations are in nanogams per liter (ng/L).

Active Ingredient				**Total Neonic. (ng/L)[1]**		**Imidacloprid (ng/L)**		**Thiamethoxam (ng/L)**		**Clothianidin (ng/L)**		**Acetamiprid (ng/L)**	
Spring 2012 (pre-seeding)	**Crop**	**Wetlands (n)**	**Detection (%)**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
	Barley	28	29	5.8	41.1	ND	ND	ND	ND	3.9	39.4	0.4	5.2
	Canola	54	52	20.7	184	1.7	30.3	2.5	19.1	16.3	144	ND	ND
	Oats	15	47	5.8	21.7	ND	ND	1.3	7.0	3.6	20.0	0.4	1.8
	Peas	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Wheat	24	25	8.3	52.7	ND	ND	4.3	32.4	3.1	20.2	ND	ND
	Grassland	15	7	1.1	7.9	ND	ND	ND	ND	1.1	7.9	ND	ND
Overall		**136**	**36**	**8.3**	**184**		**30.3 (2%)**		**32.4 (10%)**		**144 (36%)**		**5.2 (1%)**
Summer 2012 (growing)	**Crop**	**Wetlands (n)**	**Detection (%)**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
	Barley	18	83	78.9	322	1.5	18.3	19.3	91.3	57.8	277	ND	ND
	Canola	61	70	185	3110	1.8	67.9	40.3	1490	142	3110	1.1	54.4
	Oats	3	100	131	235	ND	ND	121	234	9.4	27.0	ND	ND
	Peas	8	50	9.6	28.4	ND	ND	ND	ND	9.6	28.4	ND	ND
	Wheat	29	62	53.5	524	15.9	256	2.3	37.7	35.0	518	ND	ND
	Grassland	15	13	2.7	5.8	ND	ND	ND	ND	0.8	4.1	0.4	2.3
Overall		**134**	**62**	**76.8**	**3110**		**256 (8%)**		**1490 (19%)**		**3110 (51%)**		**54.4 (1%)**
Fall 2012 (harvest)	**Crop**	**Wetlands (n)**	**Detection (%)**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
	Barley	13	8	1.1	7.0	ND	ND	ND	ND	1.1	7.0	ND	ND
	Canola	35	20	5.4	32.6	ND	ND	2.2	20.0	2.0	30.9	0.6	11.8
	Oats	3	33	4.2	12.0	ND	ND	ND	ND	ND	ND	4.2	12.0
	Peas	5	40	5.3	16.0	ND	ND	3.6	14.6	ND	ND	0.5	1.6
	Wheat	15	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Grassland	9	22	13.5	101	ND	ND	11.9	100	ND	ND	0.4	2.0
Overall		**80**	**16**	**4.0**	**101**		**ND (0%)**		**100 (6%)**		**30.9 (5%)**		**12.0 (5%)**
Spring 2013 (pre-seeding)	**Crop**	**Wetlands (n)**	**Detection (%)**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**	**Mean**	**Max**
	Barley	16	94	74.9	212	ND	ND	19.8	107	53.2	157	ND	ND
	Canola	51	98	53.1	178	1.4	4.8	12.6	93.5	38.5	173	ND	ND
	Oats	3	100	60.7	102	ND	ND	41.9	79.4	16.9	20.4	ND	ND
	Peas	6	100	33.3	60.6	ND	ND	ND	ND	33.3	60.6	ND	ND
	Wheat	9	89	41.4	85.3	ND	ND	18.2	58.2	21.4	30.7	ND	ND
	Grassland	5	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Overall		**90**	**91**	**52.7**	**212**		**4.8 (2%)**		**107 (23%)**		**173 (87%)**		**ND (0%)**

[1] Total neonicotinoid concentrations are the sum of all four active ingredients detected in wetland samples.

NS: not sampled; wetlands for this crop were dry, absent or overflooded.

ND: not detected; LOQ: Acetamiprid (0.25 ng/L); Clothianidin (0.6 ng/L); Thiamethoxam (0.9 ng/L); Imidacloprid (0.55 ng/L).

Thiamethoxam and clothianidin were detected in all 4 sampling periods; imidacloprid was not detected in fall 2012 and acetamiprid was not detected in spring 2013. Clothianidin was the most commonly detected neonicotinoid in water samples, and had the highest maximum and mean concentrations during three of the sampling periods: spring 2012 (max: 144 ng/L; mean: 16), summer 2012 (max: 3110 ng/L; mean: 142), and spring 2013 (max: 173 ng/L; mean: 39) (Table 2.2). In the fall, thiamethoxam had the highest maximum concentration (max: 100 ng/L; mean: 12).

Differences in mean concentrations between field crop types were apparent. Wetlands situated in barley, canola and oat fields had significantly higher mean annual concentrations than those in grasslands (Table 2.3, Fig. 2.3). Pre-seeding (spring 2012) concentrations had a small ($\beta \pm$ S.E.: 0.15 ± 0.06, $P = 0.01$), but positive effect on summer 2012 concentrations whereas previous year's (2011) crop type did not (Table 2.3).

Table 2.3 Results of generalized linear mixed model analyzing total neonicotinoid concentration in response to crop type and season. Total neonicotinoid concentration was measured repeatedly in up to 136 wetlands situated on 50 agricultural quarter sections in Saskatchewan during spring 2012 through spring 2013.

Fixed Effects	**β Estimate ± SE**	***t***	***P***
(Intercept)	0.83 ± 0.54	1.53	0.13
Season (reference: summer 2012)			
Fall 2012	0.34 ± 0.57	0.60	0.55
Spring 2013	-0.12 ± 0.60	-0.21	0.84
Crop (reference: Grassland)			
Barley	2.29 ± 0.80	2.84	**0.007**
Canola	2.23 ± 0.74	3.03	**0.004**
Oats	3.43 ± 1.42	2.42	**0.02**
Peas	0.85 ± 1.00	0.84	0.41
Wheat	1.07 ± 0.73	1.47	0.15
Spring 2012 Concentration	0.15 ± 0.06	2.51	**0.014**
2011 Crop (reference: Grassland)			
Barley	-0.05 ± 0.49	-0.10	0.92
Canola	0.11 ± 0.40	0.29	0.77
Oats	0.34 ± 0.53	0.63	0.53
Wheat	0.22 ± 0.49	0.45	0.65
Season x Crop (reference: Summer 2012 Grassland)			
Barley Fall 2012	-3.04 ± 0.76	-3.98	**0.0001**
Barley Spring 2013	0.29 ± 0.75	0.39	0.70
Canola Fall 2012	-2.55 ± 0.64	-3.97	**0.001**
Canola Spring 2013	0.25 ± 0.66	0.38	0.71
Oats Fall 2012	-3.59 ± 1.39	-2.58	**0.011**
Oats Spring 2013	-0.82 ± 1.29	-0.64	0.53
Peas Fall 2012	-0.96 ± 0.97	-1.00	0.32
Peas Spring 2013	1.41 ± 0.95	1.49	0.14
Wheat Fall 2012	-1.74 ± 0.70	-2.48	**0.014**
Wheat Spring 2013	1.30 ± 0.74	1.76	0.08
Random Effects	**Variance**		**SD**
Season x Quarter Section			
Summer 2012	1.14		1.07
Fall 2012	0.13		0.11
Spring 2013	0.23		0.48
Season x Wetland (nested within Site)			
Summer 2012	1.36		1.16
Fall 2012	0.50		0.70
Spring 2013	0.52		0.72

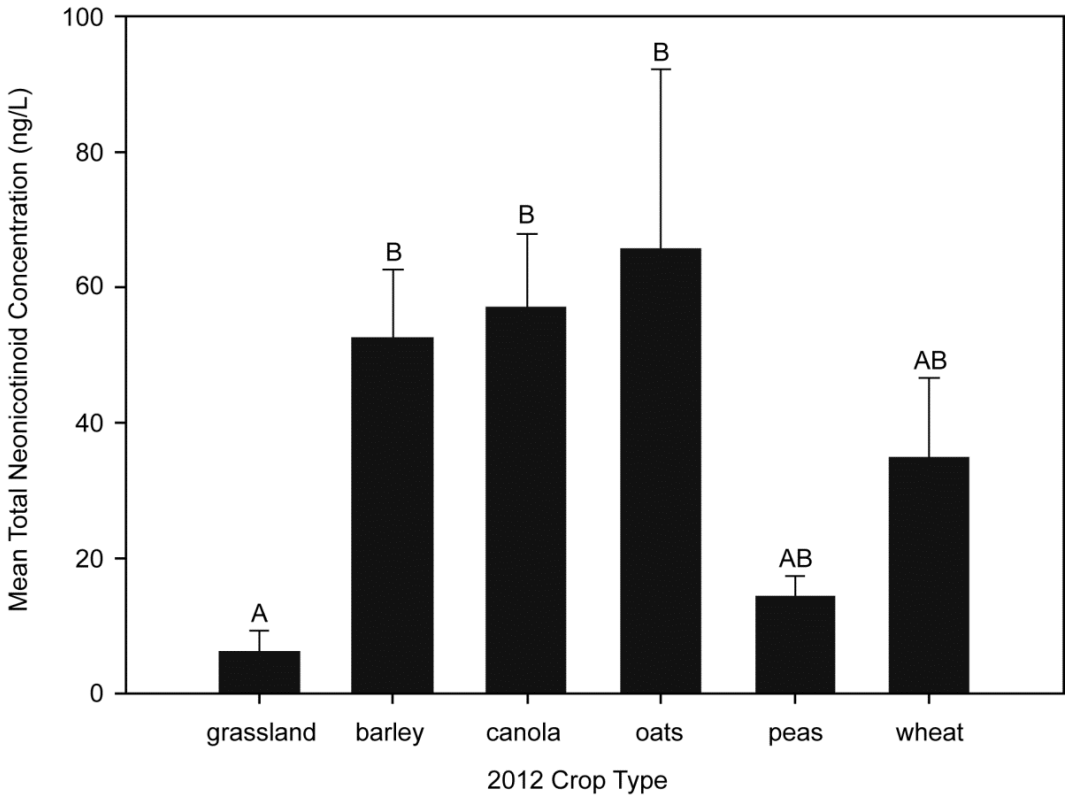

Figure 2.3 Mean total neonicotinoid water concentrations (nanograms per liter, ng/L) by crop type measured in wetlands in central Saskatchewan. Bars represent means (±SE) for each crop over all sampling periods in 2012-2013. Statistical comparisons (letters) of individual crops are relative to grasslands. Bars sharing the same letter (i.e. A, B) indicate no statistical difference in means.

Strong interactions between season and crop type (Table 2.3, Figure 2.4) masked the effect of season alone in the model. Declines in concentrations between summer and fall were found for barley (X^2 = 28.01, P = <0.0001), canola (X^2 = 55.13, P = <0.0001), and wheat (X^2 = 11.59, P = 0.0007). Subsequent increases in concentrations were found the following spring 2013 for barley (X^2 = 79.66, P = <0.0001), canola (X^2 = 150.74, P = <0.0001), wheat (X^2 = 53.48, P = <0.0001), field pea (X^2 = 11.25, P = 0.0008) and oat (X^2 = 9.75, P = 0.002) though not grasslands (X^2 = 0.86, P = 0.35).

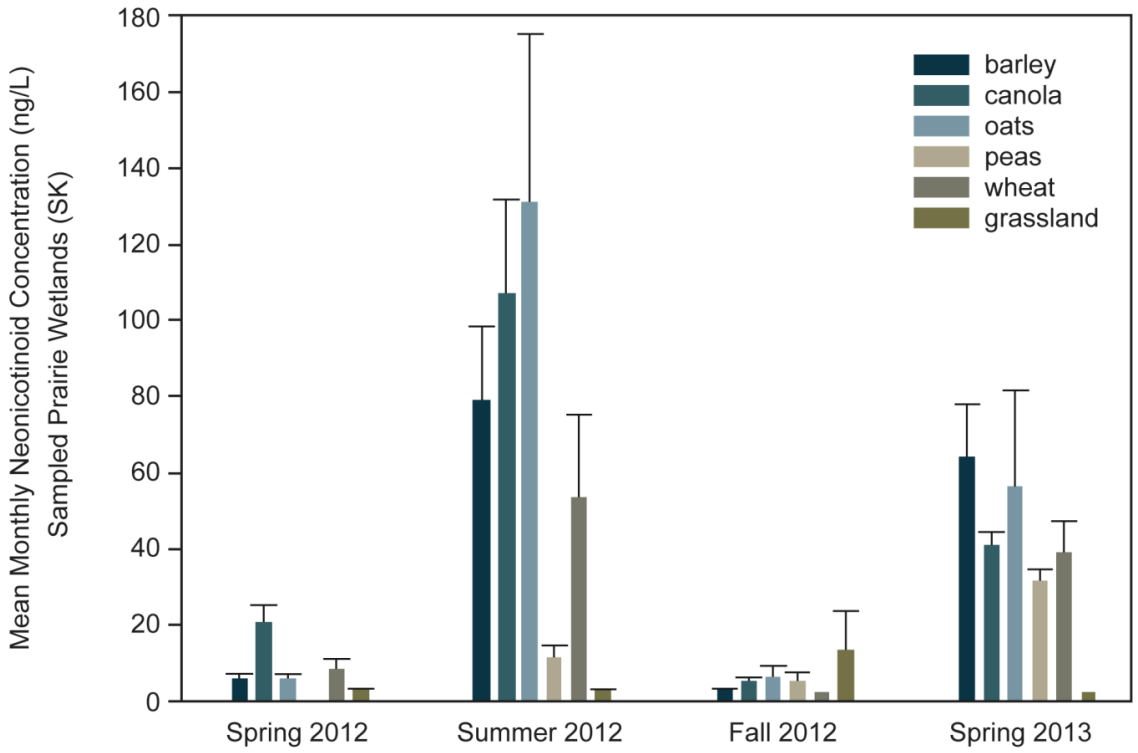

Figure 2.4 Mean total neonicotinoid water concentrations (ng/L) measured in wetlands sampled in central Saskatchewan over one year. Wetlands were sampled repeatedly over an annual growing cycle (spring 2012, summer 2012, fall 2012 and spring 2013). Spring 2012 wetlands reflect the 2011 crop type whereas summer 2012 and spring 2013 samples reflect new crops that were seeded in 2012.

Because many wetlands were dry, thus not sampled in fall 2012, the significant decline and subsequent increase in neonicotinoid concentrations between seasons could result from a sampling effect rather than a within-wetland temporal trend. However, neonicotinoid concentrations were similar between ponds that dried in the fall and those that remained wet in both summer 2012 (U(df =1) = 1558, P = 0.34) and spring 2013 (U(df =1) = 688, P= 0.99).This indicates that the observed fall decline and spring rebound in total neonicotinoid concentrations occurred within individual wetlands and was not a sampling effect.

2.4.3 Neonicotinoid residues in sediment

Of the sediment samples collected during summer 2012, only 8 (6%) of the wetlands situated in fields of barley, canola, field pea and wheat contained neonicotinoid active

ingredients (Table 2.4). The highest concentrations of each compound were thiamethoxam (max: 20.0 µg/kg, canola), imidacloprid (max: 17.5 µg/kg, canola), and clothianidin (max: 4.4 µg/kg, peas). Acetamiprid was not detected in any sediment sample.

Table 2.4 Summary of detections, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients in the sediment of 134 sampled Prairie wetlands of central Saskatchewan (summer 2012). Concentrations are in micrograms per kilogram (µg/kg).

Crop	Wetlands (n)	Detections (%)	Imidacloprid (µg/kg)		Thiamethoxam (µg/kg)		Clothianidin (µg/kg)		Acetamiprid (µg/kg)	
			Mean	Max	Mean	Max	Mean	Max	Mean	Max
Barley	18	5.6	ND	ND	ND	ND	2.6	2.6	ND	ND
Canola	61	6.6	17.5	17.5	20.0	20.0	3.4	3.9	ND	ND
Oats	3	0.0	ND	ND	ND	ND	ND	ND	ND	ND
Peas	8	12.5	ND	ND	ND	ND	4.4	4.4	ND	ND
Wheat	30	6.7	ND	ND	ND	ND	2.8	3.3	ND	ND
Grassland	14	0.0	ND	ND	ND	ND	ND	ND	ND	ND
	134	**6**	**17.5**	**17.5**	**20.0**	**20.0**	**3.3**	**4.4**	***	***

ND: indicates no detection of specific neonicotinoid active ingredient was found in the wetland sediment sampled.

2.5 Discussion

To our knowledge, this is the first study that specifically assessed the scale of use of neonicotinoids in any Canadian region and level of neonicotinoid contamination in wetlands. Sales of neonicotinoid seed treatment products in Canada have rapidly expanded since the early 2000s when seed treatments using thiamethoxam (canola, mustard) and clothianidin (canola, corn) were registered. From 2002-2005, uses of thiamethoxam further increased to include seed treatment products for wheat, barley, soybean, corn, field pea, dry bean, sunflower and lentil. Globally, uses of the neonicotinoid active ingredients examined in this study have been registered for a number of foliar, soil and seed treatment applications: imidacloprid (140), acetamiprid (60), thiamethoxam (115) and clothianidin (40) (Elbert *et al.*, 2008). The multiple seed-treatment products applied across widely distributed agricultural crops over large geographic areas presents a high degree of environmental loading and increases the potential for contamination of surface waters by neonicotinoids. According to our GIS analysis of

neonicotinoid use on the Canadian Prairies, smaller areas with high application rates appear to be in regions where corn and soybean (southeastern Manitoba) and pulses or field pea (southern Saskatchewan) are extensively seeded. Mappings created by the Pesticide National Synthesis Project of "estimated agricultural use" of clothianidin, imidacloprid and thiamethoxam revealed corresponding exponential growth throughout the United States since the early 2000s. Zones of high use (presented as pounds per square mile) are similarly located in regions growing corn, soybean and crops such as cotton (USGS, 2013). Our analysis also showed that large areas seeded to canola are treated with medium-high application rates. The same can be mentioned of cereals such as wheat and barley indicating neonicotinoid seed treatments are gaining popularity.

The number of previous studies in which surface waters (rivers, lakes and streams) in North America were monitored for neonicotinoids is generally limited (Denning, 2004; Dunn, 2004; Phillips and Bode, 2004; Xing *et al*., 2013) with only one study on wetlands (Anderson *et al*., 2013). Moreover, most studies have focused solely on the presence of imidacloprid. For example, in California, 89% of river samples had detections with concentrations of 50 to 3290 ng/L (Starner and Goh, 2012). Maximum imidacloprid values, detected in stream and agricultural run-off studies of eastern Canadian provinces (New Brunswick; Prince Edward Island), ranged from 420 ng/L to 15,880 ng/L (Denning, 2004; Dunn, 2004; Xing *et al*., 2013). Given the physico-chemical properties of neonicotinoids, they are highly susceptible to transport into aquatic ecosystems. Neonicotinoids appear to behave similarly to other pesticides which move into aquatic systems in pulses during surface run-off and deposition of aerial spray drift (Liess *et al*., 1999; Beketov and Liess, 2008). It is unclear if other factors such as wind erosion of treated seeds during spring planting also influence neonicotinoid transport into wetlands. Peak concentrations of all four neonicotinoids in the water columns of wetlands in cropped fields (not grasslands) occurred in summer 2012 with a mean concentration of 91.7 ng/L, but with maximum concentrations, which frequently consisted of more than one neonicotinoid, being as high as 3110 ng/L. However, grab sampling in rivers is known to underestimate actual maxima concentrations by 1-3 orders of magnitude and average concentrations of pesticide residues by 50% (Xing *et al*., 2013); although difficult to directly compare lentic and lotic systems, the same may be plausible of wetlands in our study area.

Our mapping of potential neonicotinoid use within the PPR based on commonly grown crops (canola, barley, wheat, oat and field pea) suggested that wetlands situated within the PPR are exposed to neonicotinoid insecticides from seed treatments. Sampling the water column of a subset (range: fall 2012 = 80; spring 2012 = 136) of wetlands within the PPR confirmed that neonicotinoids were consistently present in 16 - 91% of the monitored wetlands situated in fields seeded to canola, barley, wheat, oat and field pea and in concentrations significantly higher than those detected in comparable wetlands situated in grasslands. This may have consequences for the numerous ecosystem services provided by Prairie pothole wetlands. Wetlands not only provide functions to agricultural production (e.g., clean water for livestock), they provide habitat for a large number of species such as waterbirds, amphibians and invertebrates (Bedford *et al*., 2001; Bartzen *et al*., 2010). Importantly, a small proportion of grassland wetland samples had low levels of neonicotinoids further suggesting its susceptibility to transport and potential to affect those wetlands that are isolated from agricultural production.

While maximum neonicotinoid concentrations were typically detected in wetlands situated in canola fields, wetlands in fields seeded to other crops that were monitored in the current study were also found to contain similar mean neonicotinoid levels. This may be explained by: 1) the current high economic yield of canola, resulting in frequent 2 or 3 year rotations with wheat, barley, oat or field pea (Canola Council of Canada, 2013), 2) high soil persistence that exhibits carry over between seasons and/or 3) the area of cereal crops treated by neonicotinoids has grown exponentially since 2004 leading to higher susceptibility of wetlands to neonicotinoid contamination.

Although unexpected, we found high frequency of neonicotinoid detections prior to spring planting: 36% of 136 wetlands in spring 2012 and 91% of 90 wetlands in spring 2013. Spring water samples most commonly contained clothianidin (max = 173 ng/L) and often also contained thiamethoxam. This was despite the fact that most of the same wetlands the previous autumn had no detectable concentrations of neonicotinoids and they were not strongly retained in wetland sediments. Neonicotinoids have relatively low soil-water organic carbon partition coefficients (K_{oc}) and high water solubility (e.g., clothianidin log K_{oc} = 123, solubility = 327 mg/L) thereby limiting the potential for retention and accumulation in wetland sediments (HSDB, 2012). Clothianidin (DT_{50}= 148 – 1,155 d) and thiamethoxam (DT_{50}= 51 d) are highly

persistent in soil (HSDB, 2012) with higher reported DT_{50} values likely reflecting cold soil temperatures as frequently encountered in the Canadian Prairies . This is in agreement with regulatory studies indicating that clothianidin soil half-lives (DT_{50} values) were 385 d in Ontario, but 1386 d in North Dakota (PMRA, 2004). In support, a Saskatchewan study similarly found 80% of the initial (0-day) concentration in soil was still present after 775 d, indicating extremely high persistence in soils under Prairie conditions (PMRA, 2004). We speculate that neonicotinoid concentrations detected in Prairie wetlands in spring 2012 and 2013 were not due to persistence in water or sediment, but resulted from carryover in the soil during winter and subsequent transport to the wetlands in snowmelt runoff.

Continuous low-level contamination of wetlands by neonicotinoids both early and mid-season may have important implications for insect emergence patterns since chronic, low-level exposure may reduce invertebrate survival and growth (Alexander *et al*., 2007; Stoughton *et al*., 2008). A recent study of macroinvertebrate decline in Dutch surface waters found a significant negative relationship between imidacloprid concentrations and abundance of aquatic macroinvertebrates (Van Dijk *et al*., 2013). Field studies and studies of sublethal insect toxicity from chronic exposure are generally scarce. However, the results of the current study show these compounds are continuously detected in wetlands over several months. Prolonged exposure of invertebrates to the neonicotinoids as a result of persistence, or repeated pulses to the wetlands as documented here likely lowers the dose required to cause toxicity over short-term exposure (Beketov and Liess, 2008; Stoughton *et al*., 2008; Tennekes and Sanchez-Bayo, 2011). In addition, we detected more than one neonicotinoid in many wetlands; therefore, it is equally important to understand the cumulative effects of long-term exposure to mixtures of neonicotinoids and the potential for additive or synergistic effects of multiple neonicotinoids on aquatic organisms. Investigating single-pulse exposure of thiacloprid to stream invertebrate communities, Beketov *et al*. (2008) found that short-living species recovered after 10 weeks of contamination whereas long-living invertebrate species did not recover until almost 7 months later. Furthermore, in the current study, peak concentrations were detected during summer months when insect emergence patterns show greatest plurality suggesting food web effects may be significant.

In Canada, an interim water quality guideline for regulation of imidacloprid for the protection of freshwater aquatic life is set at 230 ng/L (CCME, 2007). Other guidelines for imidacloprid have been set by the U.S. EPA at 1050 ng/L for long term exposure and 35,000 ng/L for acute pulse events (US EPA, 2010). The European Water Framework Directive applies a Maximum Permissible Concentration (MPC) of 65 ng/L for long term exposure or Maximum Acceptable Concentration (MAC) of 200 ng/L for acute exposure. In regard to thiamethoxam, the US EPA has a published guideline for acute exposure set at 17,500 ng/L. Recently, Mineau and Palmer (2013) recommended 10-30 ng/L as a protective concentration under long term exposure based on a species sensitivity distribution analysis and the HC_5 using available chronic toxicity studies (American Bird Conservancy, 2013). The mean and maximum concentrations of clothianidin and thiamethoxam detected in this study frequently exceeded many of these guidelines based on the chemically related compound, imidacloprid. For example, clothianidin was detected at concentrations up to 14 times above the modest Canadian benchmark for imidacloprid. However, this must be interpreted cautiously because there are currently no accepted aquatic benchmarks for either of clothianidin or thiamethoxam in Canada and most international regulatory agencies are currently reviewing their existing guidelines.

2.6 Conclusion

Modelling neonicotinoid seed treatment applications within the PPR in Canada revealed increasing use over a large geographic area. Due to the intensity of crop rotations with neonicotinoid treated crops and the high environmental persistence of neonicotinoids in soil, the potential for environmental loading and transport into wetlands appears high. Monitoring the water column of a subset of wetlands within the PPR in Saskatchewan confirmed that neonicotinoid insecticides were repeatedly present in many of the wetlands sampled. Our findings have important implications for wetland ecosystem services such as litter breakdown, nutrient cycling and aquatic insect production, with potential consequences for wetland dependent species (e.g., amphibians, waterfowl; aerial insectivorous birds). In order to fully understand the effects of neonicotinoids on PPR wetlands, we recommend future studies: 1) determine levels of neonicotinoid contamination in other regional aquatic systems and across a landscape level scale; 2) determine the ecological features that make PPR wetlands susceptible to

neonicotinoid contamination; and 3) identify insect abundance, productivity and emergence responses to chronic and repeated neonicotinoid exposures.

2.7 Acknowledgements

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PREFACE TO CHAPTER 3

During the growing season, neonicotinoid insecticides are frequently transported to surface water systems after rainfall events. However, in my previous study I found detectable levels of neonicotinoids in wetlands during early spring, after ice-off but before crop seeding had begun. This may represent an unexpected long-term exposure risk for aquatic organisms. Neonicotinoid presence in early spring also suggests long-term persistence, though origins and transport mechanisms remain unknown. Therefore, the objectives of Chapter 3 were to: 1) identify the major source of neonicotinoids to wetlands in spring; and, 2) examine what factors affect change in spring wetland neonicotinoid concentrations over time.

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*Minimal changes to the original published manuscript are reflected in the subsequent text including some clarification of overall text and formatting of the references.

CHAPTER 3: SNOWMELT TRANSPORT OF NEONICOTINOID INSECTICIDES TO CANADIAN PRAIRIE WETLANDS

3.1 Abstract

During the growing season, neonicotinoid insecticides are frequently transported to surface water systems after rainfall events. However, detectable levels of neonicotinoids have also been found in wetlands during early spring, after ice-off but before crop seeding, representing an unexpected long-term exposure risk for aquatic organisms. This suggests long-term persistence, though origins and transport mechanisms remain unknown. We sampled 16 agricultural fields in the Canadian Prairies to investigate whether snow meltwater, particulate matter, top- (15 cm) or bottom-layer (15 cm) snow were potential sources of spring neonicotinoid contamination to receiving wetlands. Agricultural fields were selected based on the previous year's crop: eight canola fields (clothianidin-treated seed) and eight oat fields (un-treated). We further sampled the wetlands draining those same oat and canola fields from ice-off to seeding to assess changes in neonicotinoid concentrations over time. Top-layer snow was below the limit of quantification for both canola and oat fields. Neonicotinoid concentrations (sum of clothianidin and thiamethoxam) were highest in meltwater (canola, mean: 267 ± 72.2 ng L^{-1}; max: 633), but also detected in bottom-layer snow (oat, mean: 36.1 ± 9.18 ng L^{-1}; max: 92.9), and particulate matter (canola, mean: 10.2 ± 1.82 µg/kg; max: 17.2). Meltwater showed a stronger relationship (R^2 = 0.35) with initial neonicotinoid concentrations in wetland water than any other source type. Temporary wetland hydrology is largely fed by meltwater thus spring total neonicotinoid concentrations were higher in temporary wetlands than seasonal/semi-permanent wetlands (P = 0.003). Only clothianidin was detected in soil particulate matter samples, including from oat fields not treated the year before, confirming this compound can persist over multiple years under local field conditions. The results of this study suggest that under normal agricultural practices, wetlands in colder climates are likely to be contaminated even before seeding occurs through persistence of neonicotinoids in soil and transport of snowmelt and particulate to surface water during spring runoff.

Keywords: wetlands, insecticide transport, persistence, snowmelt, clothianidin, thiamethoxam

3.2 Introduction

Canada is home to an estimated 127 million ha of wetlands, accounting for 25% of the world's wetland area (Government of Canada, 1991). In the Prairie region, wetlands are often situated in productive farmlands where agricultural activities (e.g., drainage, agrochemical use) affect almost all wetlands directly or indirectly through mechanisms including increased siltation and destruction of wetland plants by herbicides (Kantrud *et al.*, 1989; Bartzen *et al.*, 2010). Agricultural wetlands are sometimes viewed as non-productive acreage (Wrubleski and Ross, 2011), but these surface water systems provide a suite of critical ecosystem services: water filtration, flood attenuation, and habitat and food resources for wetland-dependent organisms (e.g., birds, amphibians; Taft and Haig, 2005; Zhang *et al.*, 2007). Production of diverse aquatic prey resources is especially critical during early spring staging and breeding periods for migratory insectivorous birds, waterfowl and shorebirds (Swanson *et al.*, 1985; Davis and Smith, 2001; Mengelkoch *et al.*, 2004; Baschuk *et al.*, 2012).

More recent agrochemical threats to wetlands are the neonicotinoids, which are the fastest growing class of insecticides in modern crop protection (Jeschke *et al.*, 2010). Neonicotinoids (e.g., acetamiprid, clothianidin, imidacloprid, and thiamethoxam) are frequently used as seed treatments on major agricultural crops across North America and Europe, including canola, cereals (e.g., wheat), corn and soybeans (Elbert *et al.*, 2008). Clothianidin, imidacloprid, and thiamethoxam combined are registered for 295 crop uses in 120 countries (Jeschke *et al.*, 2010). Although most seed treatments pre-date the advent of neonicotinoids (Buttress and Dennis, 1947), the large-scale production and mono-cropping common in current agricultural practices has led to over 95% of canola seeds being treated with some type of neonicotinoid active ingredient in Canada (Main *et al.*, 2014), and 79-100% of corn in the United States (Douglas and Tooker, 2015). Pesticide sales data in Canada is considered confidential and seed-applied pesticide products were not accounted for by the National Agricultural Statistics Service major use survey in the United States, but neonicotinoid seed treatments continue to grow in popularity and spatial extent (USGS, 2012; Main *et al.*, 2014; Douglas and Tooker, 2015). Insecticide applications typically extend throughout the Great Plains region – including the Canadian Prairies – which often directly overlaps with high density wetland environments.

Neonicotinoid seed treatments are designed to protect the individual plant while greatly reducing the amount of insecticide used in subsequent spray or soil drenching applications, thus theoretically lessening impacts on the environment (Elbert *et al*., 2008; Jeschke *et al*., 2010). However, typically 5% (max 20%) of the active ingredient may be absorbed by the target crop (Sur and Stork, 2003), while the remaining active ingredient may remain in soils (Goulson, 2013). Estimates of clothianidin and thiamethoxam persistence in soils are variable with clothianidin persisting in fields (half-life: DT_{50}) from 277 to 1386 days (DeCant and Barrett, 2010), and thiamethoxam persisting in fields for 7 to 109 days (Goulson, 2013). Neonicotinoids also accumulate in soils over time (Bonmatin *et al*., 2005b; Jones *et al*., 2014) and are expected to persist longer in colder regions at mid to higher latitudes due to lower temperatures and lower sunlight intensity (Bonmatin *et al*., 2015). Indeed, a previous Saskatchewan field study demonstrated little to no measurable dissipation in soils over 775 days (PMRA, 2004). Additionally, both insecticides are highly water soluble (clothianidin = 327 mg/L; thiamethoxam = 4,100 mg/L) with high potential for transport into water bodies (HSDB, 2012). Neonicotinoids have been detected across a range of agricultural surface water systems in North America including rivers, streams and wetlands with peak neonicotinoid concentrations typically detected after seeding or during the growing season following rainfall events (Starner and Goh, 2012; Anderson *et al*., 2013; Hladik *et al*., 2014; Main *et al*., 2014; Smalling *et al*., 2015). However, traditional studies reporting water concentrations have rarely assessed whether contamination persists outside the growing season.

In the Canadian Prairie region, 80-85% of water stored in wetlands comes from the shallow snowpack which is highly susceptible to wind erosion or redistribution (Gray, 1970). Prairie soils can also freeze to depths of 1 m or more (van der Kamp *et al*., 2003). As the infiltration capacity of frozen soils is limited (Granger *et al*., 1984), a large amount of surface meltwater can be generated in a short amount of time during spring snowmelt which flows into depressions forming small wetlands (Hayashi *et al*., 2003). During the melt process, soil particles may be picked up and transported with the runoff (Gray, 1970). Our previous studies found that after ice-off (i.e., opening of surface water) and before seeding has occurred, between 36% (2012) and 91% (2013) of Prairie wetlands contained at least one neonicotinoid insecticide (Main *et al*., 2014) at total concentrations up to six times greater than those which may induce chronic effects to sensitive aquatic insects (Morrissey *et al*., 2015). Consequently, snowmelt

transport of neonicotinoids to wetlands, during a critical spring period, may have important implications for wetland biota.

Although we previously reported pre-seeding neonicotinoid concentrations in Prairie wetlands, the origin of those spring detections remained unknown. Therefore, our objectives were to 1) identify the major source of neonicotinoids to wetlands in spring; and, 2) examine what factors affect change in spring wetland neonicotinoid concentrations over time. We hypothesized that pre-seeding wetland water contamination originated either from snow contact with fields or soil particulate matter containing residual neonicotinoid active ingredients which may be scoured and transported to wetlands during seasonal snowmelt runoff.

3.3 Methods

We conducted fieldwork during April and May of 2014 at 16 agricultural fields (65 ha) near Alvena, Saskatchewan (52.5167° N, 106.0167° W; Fig. 3.1).

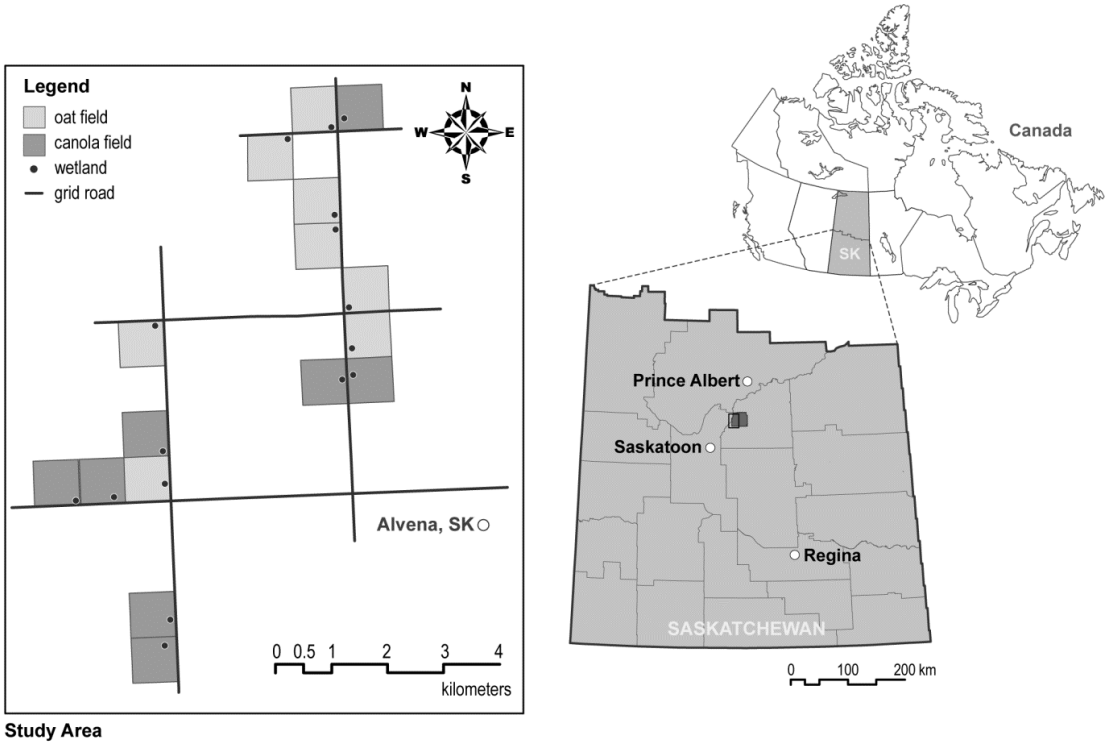


Figure 3.1 Map of agricultural study sites (untreated oat and neonicotinoid treated canola fields) located near Alvena, Saskatchewan (Canada). A single wetland was sampled within each study field (= quarter section measuring 65 ha).

Saskatchewan is characterized by warm, dry summers (e.g., daily average, July: 18.5 °C) and cold, dry winters (e.g., daily average, January: -15.5 °C); average precipitation is approximately 276.7 mm of rainfall and 91.3 cm of snow (Environment Canada, 2010). Our study fields were situated in the Black soil zone (Order: Chernozemic) which is typically associated with shorter growing periods and lower temperatures, but increased moisture leading to a wider variety of potential crops in production (average soil organic matter = 4.5 to 5.5%; SCWG, 1998). Typical crop rotation in this area of Saskatchewan is an alternating canola/cereal rotation where the majority of canola is treated with clothianidin or thiamethoxam products while a small but growing fraction of cereals are treated. We controlled for previous crop type (i.e., crop planted in spring 2013) by selecting an even distribution of fields previously seeded to either canola or oat crops. All oat fields were planted with untreated seed in the year prior (2013), whereas canola fields were previously planted using clothianidin-treated seeds (Prosper®, Bayer CropScience) at standard application rates. It should be noted that in years prior to our study (2011 and 2012), 20% of landowner canola fields were planted with thiamethoxam-treated seeds (Helix Xtra®, Syngenta). Study wetlands (n = 16; one per agricultural field) spanned a range of classes (defined by Stewart and Kantrud, 1971) including: temporary (n = 6), seasonal (n = 7) and semi-permanent (n = 3). All wetlands were <1 ha in size, ranged in initial depth from 20 cm to over 1 m and were randomly chosen based on consistent timing of availability after ice-off.

3.3.1 Snow and meltwater collection

Our snow sampling technique was modified from previously published methods (McConnell *et al*., 1998; Hageman *et al*., 2006). Top- and bottom-layer snow samples were collected using a stainless steel spade that was rinsed thoroughly between samples with deionized water. Top-layer samples were extracted from a ~15 cm x 15 cm x 30 cm core at three random points surrounding the study wetlands (~ 3 m from the wetland edge). The top ~5 cm were removed to lessen the possibility of atmospheric deposition or wind-scoured soil that may contaminate the perceived "clean layer". An additional three bottom-layer snow samples were collected at the same locations from the ~15 cm of snow that was in direct contact with the soil. All snow samples were transferred to individual polyethylene bags, transported to the lab in the dark in coolers, and immediately placed in a freezer at -20°C until analysis. Prior to analysis, snow samples were placed in a large stainless steel basin, covered and allowed to melt overnight

at room temperature (McConnell *et al*., 1998). Composite top- and bottom-layer samples were created from the three sample points and poured into chemically-cleaned (acetone: hexane) 1 L amber glass bottles before neonicotinoid extraction. Bottles were sealed with Teflon-lined caps and then refrigerated at 4°C until analysis ~ 10 days later.

Where possible, meltwater was collected from ~5 to 10 snowmelt channels evenly distributed around the study wetlands. However, due to uneven seasonal snowmelt patterns, the number of available meltwater channels surrounding the study wetland often varied (~10-20 channels) among field sites. Meltwater was drawn from the snowmelt channels using 50 cc disposable syringes and placed in a chemically-cleaned 1 L amber glass bottle. Up to ~100 ml of meltwater was collected at each channel and compiled into an overall composite sample (min. 500 ml per site). Particulate soil samples were collected by centrifuging the meltwater samples at 5000 rpm for 15 min to isolate the solid fraction for separate analysis. Composite sample bottles were sealed with Teflon-lined caps, stored in the dark during transport and then refrigerated at 4°C until analysis. Although we were interested in collecting and analyzing ice samples within the depression prior to melt, the majority of wetland basins in our study were formed by annual snowmelt and therefore ice cores were unavailable for collection.

While we had collected samples of meltwater, top- and bottom-layer snow, and particulate matter from all 16 study sites, we were unable to analyze two particulate matter samples (oat fields = 2) due to insufficient mass of particulate in meltwater solution.

3.3.2 Water sampling

Beginning six days after initial ice-off (April 16), we collected water samples on a weekly basis from each of the 16 agricultural wetlands until seeding began (approximately May 23). All sites were accessed on foot by wading into the wetland and a sub-surface grab sampling technique was used to collect wetland water 10 cm below the surface of the central portion of the pond. Water was collected using a 1 L chemically cleaned (acetone: hexane washed) amber glass bottle. Bottles were sealed with Teflon-lined caps, stored in the dark during transport and then refrigerated at 4°C until analysis.

3.3.3 Neonicotinoid analysis

All snow, meltwater, wetland water, and particulate matter samples were analyzed for 4 different neonicotinoids at the National Hydrology Research Centre, Environment Canada, Saskatoon, Saskatchewan using methods described in Main *et al*. (2014). Mean limits of quantification (LOQ) in snow, meltwater and wetland water were as follows: imidacloprid, 2.3 ng/L; thiamethoxam, 5.6 ng/L; clothianidin, 3.3 ng/L; and, acetamiprid, 1.4 ng/L. Mean recoveries from river water (n=4) fortified at 50 ng/L were as follows (mean ±SD): imidacloprid, 83.9 ± 6.74%; thiamethoxam, 95.8 ± 11.9%; clothianidin, 73.4 ± 8.69%; and, acetamiprid, 85.9 ± 7.91%. LOQ in particulate matter were as follows: imidacloprid, 1.9 µg/kg; thiamethoxam, 5.3 µg/kg; clothianidin, 2.0 µg/kg; and, acetamiprid, 0.4 µg/kg. LOQs for sediment were much higher than water due to the limited amount of sediment available for extraction and concentration. Mean recoveries from sediment fortified at 20 µg/kg (n=2) were as follows: imidacloprid, 84.6 ± 3.0%; thiamethoxam, 88.1 ± 5.6%; clothianidin, 84.8 ± 2.7%; and, acetamiprid, 84.8 ± 2.0%. All neonicotinoid concentrations were recovery corrected between batches and all laboratory and field blanks were below the limit of detection.

3.3.4 Statistical analyses

Only two (clothianidin and thiamethoxam) of the four neonicotinoids analyzed were detected in the various samples. As numerous snow (19%), meltwater (50%) and wetland water (46%) samples contained both clothianidin and thiamethoxam, we summed these concentrations and present them as total neonicotinoid concentrations for statistical analysis. Under field conditions, thiamethoxam may be degraded to clothianidin, and these chemicals are predicted to have additive effects on biota (Simon-Delso *et al*., 2015).

Effects of previous crop, wetland depth, and time on wetland neonicotinoid concentrations

We analyzed the effects of previous crop type, wetland class and sampling date on change of total neonicotinoid concentration in wetlands over time using a Gaussian general linear mixed model (GLMM) in package “nlme” (Pinheiro *et al*., 2014) in R 3.1.1 (R Core Team, 2014). We split the wetlands into temporary and seasonal/semi-permanent wetland classes based on initial central depth (Driver and Peden, 1977; Euliss and Mushet, 1996). We predicted that as initially shallow ponds increased in wetland volume, concentrations would increase due to

inclusion of greater source meltwater. Previous crop type, wetland class, and time (measured as Julian date since January 1) were fixed effects and wetland ID was included as a random effect to account for repeated measures. Model fit was assessed through a combination of visual inspection of residuals and a Shapiro-Wilk test to assess the fit of residuals to a normal distribution (Michel, 2014). We further assessed temporal autocorrelation in our repeated measures model by calculating partial autocorrelation functions, and we allowed variances to differ between dates to account for heterogeneity in model residuals (Michel, 2014). We selected fixed and random effects using Akaike's information criteria (AIC; Akaike, 1987) in an exhaustive model selection framework.

Differences in neonicotinoid concentration between crops and snowmelt source types

We analyzed the effects of previous crop type (canola, oat) and source type (top-layer/bottom-layer snow and meltwater) on total neonicotinoid concentration in snow sources using a general linear mixed model. Total neonicotinoid concentration was log transformed and analyzed using a Gaussian distribution; previous crop and source type were fixed effects, and site was included as a random effect. To improve model fit by accounting for heterogeneity we allowed variances to differ between crop types. *Post-hoc* testing to investigate interactions between source types was completed using Tukey tests corrected for multiple comparisons in package "multcomp" (Hothorn *et al*., 2013). Model fit was assessed as described above, using a combination of visual and statistical evaluation of residuals. Exhaustive model selection based on AIC was completed as described above.

As particulate matter was not a liquid source, and analyzed as concentration per weight of material rather than volume of water, it was excluded from the snowmelt model. Instead, we developed a binomial generalized linear mixed model in package "lme4" (Bates *et al*., 2014) with neonicotinoid presence/absence as the response variable. The model was otherwise identical to the neonicotinoid concentration model (above). In this model, source type included all snowmelt sources and particulate matter extracted from meltwater. However, our models did not converge due to lack of variation between source types.

Relationship between source and initial wetland neonicotinoid concentrations

We analyzed the relationship between neonicotinoid concentrations in snowmelt source (top- and bottom-layer snow, meltwater and particulate matter) and initial total neonicotinoid concentration in study wetlands using a Gaussian general linear model. Previous crop type and weekly wetland depth measurements were included as covariates. To allow for direct comparison of regression coefficients among predictors measured on different scales (orders of magnitude difference between snow and particulate sources), we standardized the predictors by subtracting the mean and dividing by the standard deviation (i.e., z-scoring; Michel, 2014). We checked for collinearity among predictor variables using Pearson product-moment correlations (between continuous predictors) and polyserial correlations (between the categorical predictor (previous crop type) and continuous predictors) in package "polycor" in R (Fox, 2010). All predictor variables had pairwise correlations of $r < 0.7$, suggesting minimal collinearity (Dormann *et al*., 2013). Model fit was assessed as described above.

3.4 Results

3.4.1 Effects of previous crop, wetland depth, and time on wetland neonicotinoid concentrations

We collected 16 water samples weekly from our study agricultural wetlands (n = 16) over five weeks between ice-off and seeding (total = 76 samples). Of the 16 wetlands, two wetlands (1 canola, 1 oat) dried two weeks before the end of our study. Clothianidin was detected in 96% (73/76) of total wetland water samples and thiamethoxam was detected in 43% (33/76) of the same water samples. Regardless of wetland class, wetlands found in previously treated canola fields contained low levels of thiamethoxam, clothianidin, or total summed neonicotinoids (Table 3.1). Wetlands in the untreated oat fields contained similar concentrations of thiamethoxam, clothianidin, or total summed neonicotinoids (Table 3.1).

Table 3.1 Summary of detections, arithmetic means (± S.E.) and maximum concentrations of summed total neonicotinoids, clothianidin, and thiamethoxam in wetland water and in source samples (top- and bottom-layer snow, meltwater, and particulate matter) collected around study wetlands in spring of 2014. Concentrations are presented in nanograms per liter (ng/L). N/D = not detected.

			Total Neonics. (ng/L)		Clothianidin (ng/L)		Thiamethoxam (ng/L)	
Sample Type	**Crop (2013)**	**Detection (%)**	**Mean ± S.E.**	**Max.**	**Mean ± S.E.**	**Max.**	**Mean ± S.E.**	**Max.**
Wetland Water[1]	Oat	100	65.9 ± 14.1	204	50.1 ± 6.55	137	18.1 ± 3.07	32.2
n = 16	Canola	95	58.0 ± 7.66	121	31.2 ± 3.47	92.5	27.0 ± 5.19	76.1
Top-Layer Snow	Oat	0	N/D	N/D	N/D	N/D	N/D	N/D
n = 16	Canola	0	N/D	N/D	N/D	N/D	N/D	N/D
Bottom-Layer Snow	Oat	100	36.1 ± 9.18	92.9	34.7 ± 9.26	92.9	11.5	11.5
n = 16	Canola	100	31.9 ± 9.17	89.1	23.9 ± 9.68	46.5	28.8 ± 13.7	42.6
Meltwater	Oat	100	181 ± 51.5	514	170 ± 49.5	489	31.4 ± 8.95	59.2
n = 16	Canola	100	267 ± 72.2	633	221 ± 42.9	385	114 ± 48.2	355
Particulate Matter[2]	Oat	100	9.91 ± 1.55	17.1	9.91 ± 1.55	17.1	N/D	N/D
n = 14	Canola	75	10.2 ± 1.82	17.2	10.2 ± 1.82	17.2	N/D	N/D

[1] A total of 76 water samples were collected from the 16 study wetlands over a five week period.

[2] Particulate matter concentrations are measured in µg/kg.

Our best model explained almost half of the variation in spring changes in wetland concentration (Table 3.2; $R^2 = 0.48$).

Table 3.2 AIC model selection table for analysis of total neonicotinoid concentration change over time in Prairie wetlands by previous crop type (canola, oat), wetland class depth (bin depth) and sampling period (Julian date). AIC values from generalized linear mixed models are shown. The best model is indicated in bold text indicating the time and wetland class interaction is the best explanatory variable.

Model	AIC
Previous Crop x Time + Wetland Class x Time + Previous Crop x Wetland Class	136.7
Previous Crop x Time + Wetland Class x Time	135.1
Previous Crop x Time + Previous Crop x Wetland Class	138.7
Wetland Class x Time + Previous Crop x Wetland Class	136.4
Previous Crop x Time + Wetland Class	140.4
Wetland Class x Time + Previous Crop	130.6
Time x Wetland Class	**128.6**
Previous Crop + Time + Wetland Class	138.5
Previous Crop + Time	139.1
Previous Crop + Wetland Class	136.8
Time + Wetland Class	136.5
Previous Crop	137.4
Time	137.7
Wetland Class	134.8
Null	136.5

Temporary wetlands were associated with higher total neonicotinoid concentrations than seasonal/semi-permanent wetlands ($\beta = 3.87 \pm 1.34$, $t = 2.89$, $P = 0.01$). There was a significant interaction between wetland class and time ($\beta = -0.03 \pm 0.01$, $t = -3.47$, $P = 0.001$), such that total neonicotinoid concentrations increased over time in temporary wetlands, but decreased slightly in seasonal/semi-permanent wetlands (Fig. 3.2). An initially sharper decrease in thiamethoxam (Fig. 3.3) may have influenced the subsequent increase of clothianidin (Fig. 3.4) over the sampling period as thiamethoxam breaks down to clothianidin.

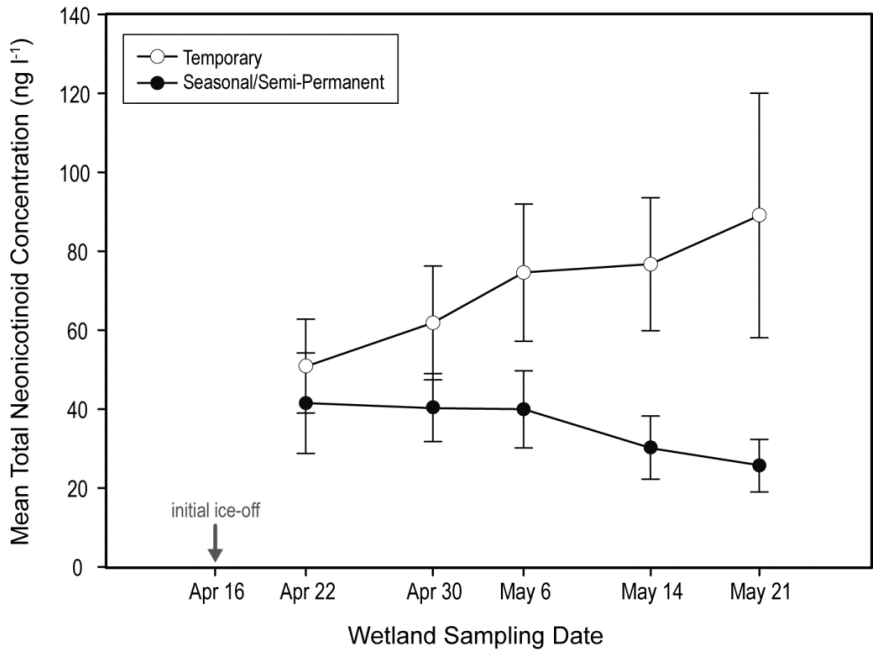


Figure 3.2 Change in mean (± SE) total neonicotinoid concentration (ng/L) in agricultural wetland water over time by wetland class. Initial ice-off date is indicated.

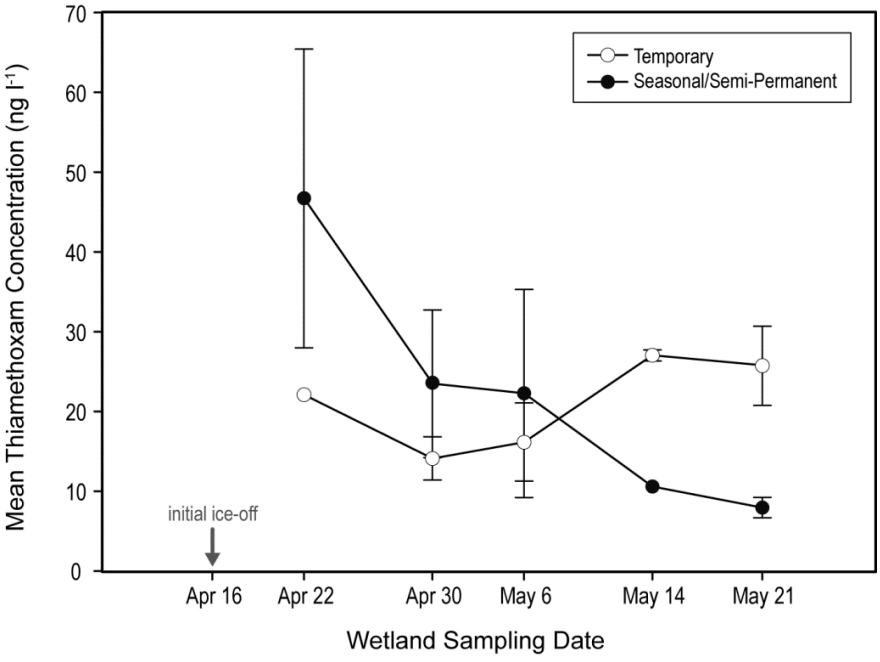


Figure 3.3 Change in mean (± SE) thiamethoxam water concentrations (ng/L) from temporary and seasonal/semi-permanent agricultural wetlands over time.

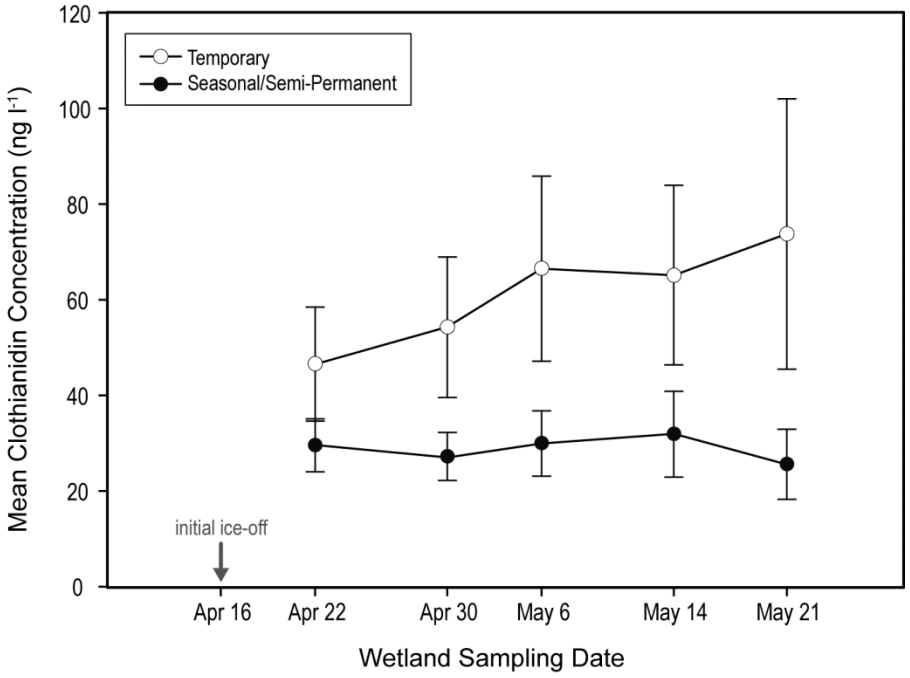


Figure 3.4 Change in mean (± SE) clothianidin water concentrations (ng/L) from temporary and seasonal/semi-permanent agricultural wetlands over time.

3.4.2 Differences in neonicotinoid concentration between crops and snowmelt source types

Fields previously planted with clothianidin-treated canola or untreated oats not only had statistically similar wetland water concentrations in spring, but also similar concentrations in the source samples (Table 3.1). All samples of top-layer snow were below detection in our study (Fig. 3.5). Neonicotinoids were detected in 100% of bottom-layer snow and meltwater, and >75% of particulate matter samples. Crop type did not influence probability of neonicotinoid detection in any source type.

Our best model indicated that there were significant differences in neonicotinoid concentration between all snow-related source types, independent of crop type (Table 3.3). Meltwater concentrations were greater than both bottom-layer snow (β = 1.74 ± 0.21, z = 8.31, P <0.0001) and top-layer snow (β = 2.79 ± 0.21, z = 13.26, P < 0.0001), and bottom-layer was greater than top-layer snow (β = 1.04 ± 0.21, z = 4.96, P < 0.0001). This model explained 76% (R^2 = 0.76) of the variance in neonicotinoid concentration.

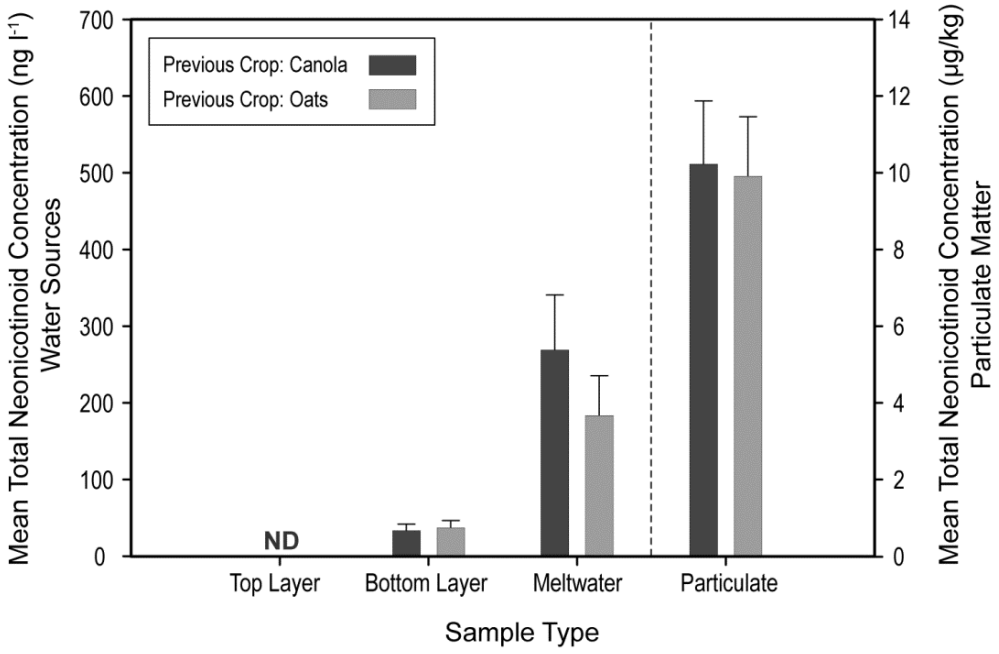


Figure 3.5 Mean (± SE) total neonicotinoid concentrations detected in spring after snowmelt by source (top- and bottom-layer snow, meltwater; ng/L) and particulate matter (µg/kg) collected from previously untreated (oat) and seed-treated (clothianidin: canola) agricultural fields in central Saskatchewan. ND = no detection.

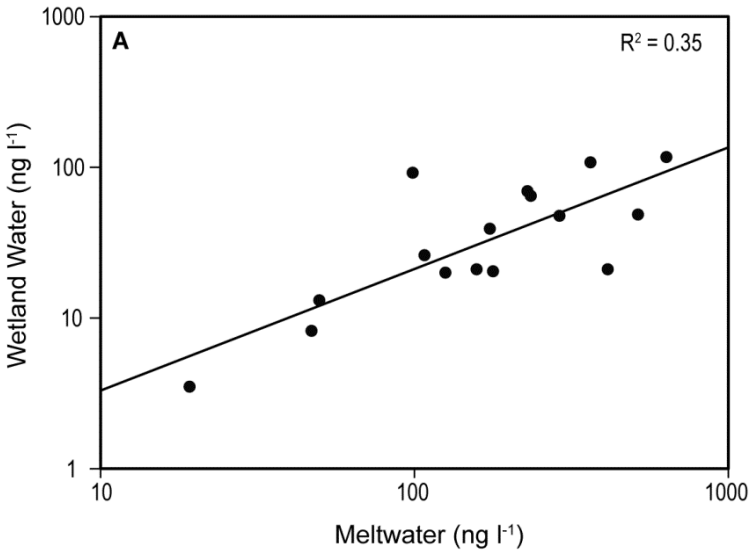
Table 3.3 AIC model selection table for analysis of total neonicotinoid concentration by previous crop type (canola, oat) and snow-related sample sources (top- and bottom-layer snow, meltwater). AIC values from general linear mixed models are shown. The best model is indicated in bold text indicating sample source was the single best explanatory variable.

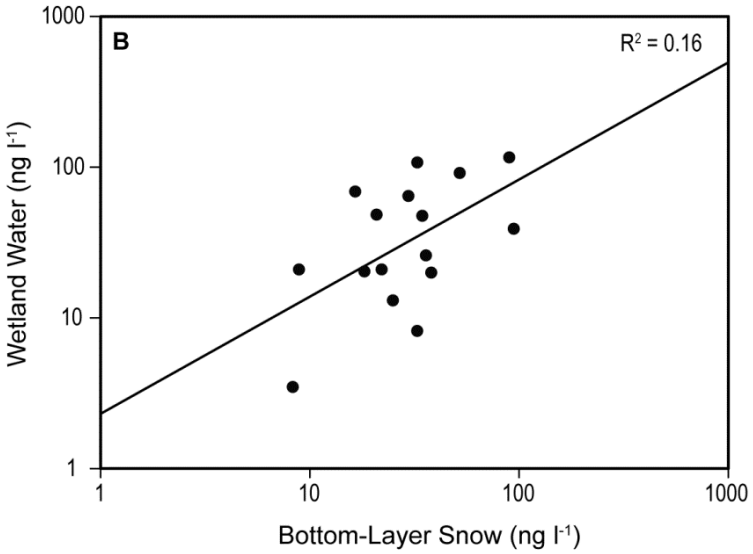
Model	AIC
Sample Source x Previous Crop	108.5
Sample Source + Previous Crop	105.5
Previous Crop	170.8
Sample Source	**103.5**
Null	168.8

3.4.3 Relationship between source and initial wetland neonicotinoid concentrations

Source sample type, crop, and wetland depth collectively explained 42% (R^2 = 0.42) of the variation in our model predicting initial neonicotinoid concentrations detected in wetland

water. All three sources contained neonicotinoids (bottom-layer snow, meltwater and particulate matter) and were positively associated with wetland concentrations. In particular, meltwater was positively associated ($\beta = 0.53 \pm 0.22$, $t = 2.42$, $P = 0.04$) with initial spring wetland concentrations (first sampling period) even after controlling for depth or surrounding crop type in the model. Meltwater also showed a stronger relationship ($R^2 = 0.35$) with initial wetland neonicotinoid concentrations than any other source type (Fig. 3.6).


A
R^2 = 0.35
Wetland Water (ng l^{-1})
Meltwater (ng l^{-1})
1000
100
10
1
10
100
1000


B
R^2 = 0.16
Wetland Water (ng l^{-1})
Bottom-Layer Snow (ng l^{-1})
1000
100
10
1
1
10
100
1000

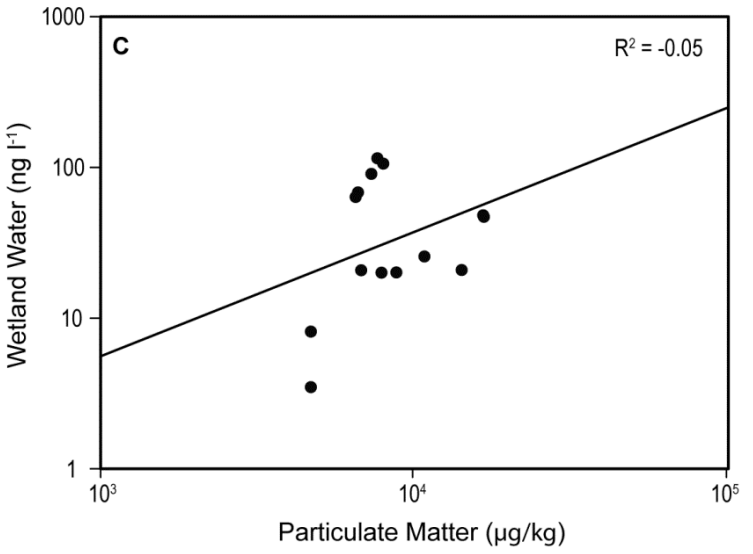

C
R^2 = -0.05
Wetland Water (ng l^{-1})
Particulate Matter (µg/kg)
1000
100
10
1
10^3
10^4
10^5

Figure 3.6 Log-log plot of mean total neonicotinoid concentrations (ng/L) in initial wetland water samples after ice-off by contributing source: A) meltwater (ng/L), B) bottom-layer snow (ng/L) and C) particulate matter (µg/kg). Adjusted R^2 values of each contributing source are indicated.

3.5 Discussion

To our knowledge, this is the first study demonstrating snowmelt in northern latitudes is a major driver of spring pre-seeding neonicotinoid contamination of surface waters. Our results are consistent with previous studies indicating that the majority (96% reported here) of Prairie wetlands sampled in spring contained neonicotinoids. Of potential sources of neonicotinoids to Prairie wetlands, meltwater had the strongest relationship with initial neonicotinoid concentrations detected in wetland water during spring, with smaller contributions from the soil particulate and soil-snow (bottom) contact layer. We further found that temporary wetlands that primarily consisted of meltwater were associated with higher neonicotinoid concentrations that increased over time. Conversely, seasonal and semi-permanent wetland classes exhibited a slight decrease in neonicotinoid concentrations over time, likely as a result of larger volume dilution. The influence of previous crop type (and its associated treatment) appeared minor suggesting persistence and carry-over of neonicotinoid residues occurs between seasons where neonicotinoid treated crops (e.g., canola) are frequently rotated with untreated crops (e.g., oats) in alternating years. Nearly half (44%) of Canadian Prairie cropland is estimated to be treated with a neonicotinoid active ingredient during a given growing season, in part due to the high frequency of canola cultivation in alternate years (Main *et al*., 2014).

In Canada, neonicotinoids have been shown to be highly persistent in soils with dissipation in the top 0-15 cm occurring slowly. Clothianidin half-life (DT_{50} or the amount of time it takes for 50 percent of the compound to disappear from soil) values in Ontario were 385 d while in Saskatchewan, a DT_{50} could not be determined by the end of a 775 d period since 80% of the initial concentration remained (PMRA, 2004). In North Dakota, with similar soils and climatic conditions, DT_{50} values reached 1386 d for clothianidin (DeCant and Barrett, 2010). Data on thiamethoxam DT_{50} values are limited with soil degradation under European field conditions ranging between 7.1 to 92.3 d for seed treatments and spray applications (Hilton *et al*., 2015). However, half-lives in laboratory soils using seed treatments have potentially higher

ranges of 46 to 3001 d indicating comparable potential for long-term persistence (Goulson, 2013).

Although we analyzed all source samples for four neonicotinoids, clothianidin was the only active ingredient detected in particulate matter samples. Particulate samples in canola fields treated in the previous year (2013) had the highest peak concentration (max: 17.2 µg/kg), but oat fields which had not seen neonicotinoid-treated seeds since 2012, overall had only slightly lower mean concentrations than canola (canola = 10.2 µg/kg; oat = 9.91 µg/kg). Based on the results of our study, it appears that neonicotinoids are more likely to persist and accumulate in areas where neonicotinoid-treated seeds are part of the normal crop rotation schedule. Persistence and accumulation in soils has been found in the United Kingdom (U.K.) where imidacloprid, clothianidin, and thiamethoxam were detected in fields without neonicotinoid seed-treatment applications in the previous three years (Jones *et al*., 2014). Consecutive treatments may also lead to higher soil concentrations than those receiving only one year of treated seed (Bonmatin *et al*., 2005b). After six years of repeated applications of imidacloprid, soil concentrations in U.K. fields ranged from 18 to 60 µg/kg one year after the final application occurred (Goulson, 2013). This effect may be exacerbated in areas and years that are receiving lower amounts of precipitation given the finding that neonicotinoid transport is primarily through water after rainfall and snowmelt.

Our findings suggest that neonicotinoid active ingredients which were likely bound to soil particles are being eroded from agricultural fields during spring seasonal freeze-thaw periods. Although snowfall is often more spatially uniform compared to rainfall, snow is highly susceptible to wind redistribution; during the melt process, snow particles often mix with soil particles. This reduces solar reflection, speeds up snowmelt and leads to earlier exposure of soil layers (Gray, 1970). As these bare soil areas warm and dry, interactions such as loosening of soil particles through wind redistribution of blowing snow (Fang and Pomeroy, 2009) or meltwater erosion (Gray, 1970) likely occur between soil-bound neonicotinoids and snowmelt water (Main *et al*., 2014). Erosion of soil particles by meltwater is highly plausible as large amounts of snowmelt water are generated in short periods of time (Hayashi *et al*., 2003). Meltwater has been previously identified as a major transport mechanism of other pesticides, especially those that are highly water soluble such as 2, 4-D and lindane (Nicholaichuk and Grover, 1983; Wania, 1997;

Donald *et al.*, 1999). We observed that fine soil particles were transported in meltwater channels within a few days of surface melt. In our study, meltwater had significantly higher concentrations (range: 14.3 to 633.3 ng/L) than other snow sources such as the top- (<LOQ) and bottom-layer snow (range: 6.6 to 92.9 ng/L). Ample quantities of particulate matter (mean ± SE: 1.2 ± 0.21g; max: 3.05) were collected in 14 of 16 meltwater samples, further supporting the explanation of soil particle erosion by snowmelt. Interestingly, the peak meltwater concentration (809 ng/L; previously canola) was opportunistically collected three weeks after initial snowmelt had occurred on a field with some remnant snow-drifts. This further suggests surface water concentrations may continue to increase over time as meltwater erodes additional soil particles or through saturation and interaction with contaminated, deeper soil layers.

Spatial distribution of snowpack in Prairie agricultural landscapes is affected by wind redistribution of snow from exposed sites to more sheltered areas, as well as topographic features and land cover roughness such as field stubble (Fang and Pomeroy, 2009). Regardless of snow depth, all top layer snow samples had neonicotinoid concentrations below the LOQ in our study. Conversely, concentrations in bottom layer snow - which interacts directly with the soil surface - ranged from 6.6 ng/L (previously canola) to 92.9 ng/L (previously oats). In a study of neonicotinoid residues in corn fields, clothianidin concentrations ranging from 20 ng/L (composite sample) to 200 ng/L (heavily soiled drifts) were detected in three snow-drift samples collected in Ontario corn fields whereas levels of six other neonicotinoids were below the detection limit (Schaafsma *et al.*, 2015). Although the Ontario corn field snow-drifts had a similar range of neonicotinoid concentrations as the layered snow samples reported here, different sampling techniques (soil-scoured snow drifts vs. individual snow layers) precludes a direct comparison of concentrations between locations and crop types.

In this study, total neonicotinoid concentrations were significantly higher in temporary wetlands, i.e., those wetlands with an initial depth <40 cm (Euliss and Mushet, 1996). Moreover, concentrations increased over time in shallow temporary wetlands, whereas they decreased slightly in deeper seasonal and semi-permanent wetlands. Although inputs of more meltwater could equate to higher neonicotinoid concentrations in deeper seasonal and semi-permanent wetlands, we found the opposite to be true. We present several plausible hypotheses for this observation. This could be due to remnant stands of vegetation or presence of ice on and around

the wetlands in late winter. Deeper wetlands are typically surrounded by a buffer of tall, coarse cattails or bulrushes (Stewart and Kantrud, 1971; Millar, 1976; Kantrud *et al*., 1989). However, vegetation such as coarse emergent species that have senesced in fall likely accumulate greater quantities of seasonal blowing snow and reduce the amount of transport or run-off (van der Kamp *et al*., 2003; Fang and Pomeroy, 2009). Deeper wetlands may also retain water and accumulate snow in the basin that freezes overwinter, while temporary wetland basins dry out before winter and are fed almost completely by meltwater in spring. Therefore, we hypothesize that these deeper wetlands are 1) receiving more "clean" snow run-off from seasonal accumulation around previous year's wetland vegetation that is not interacting with treated soils; and, 2) experiencing greater meltwater accumulation from snow directly in the wetland depression and/or ice formed from the wetland the previous year, which may then lead to stronger dilution and/or 3) are ameliorated by intact senesced plant buffers during the runoff period.

Most small wetlands across the Prairies are fed by snowmelt runoff due to frozen soils preventing infiltration (Hayashi *et al*., 2003) with up to 90% of snowmelt collecting in wetland depressions (Kantrud *et al*., 1989). We found a weak, but positive association between spring (pre-seeding) neonicotinoid concentrations in wetlands and meltwater concentrations regardless of crop type. These spring detections are notable because they are typically an indicator of wetland contamination later in the season. Neonicotinoid detections in spring-sampled wetlands were more likely to be associated with detectable and/or higher concentrations during the summer growing period (Main *et al*., 2014). Thus long-term presence of these highly toxic insecticides in water will prolong the exposure period for aquatic organisms.

Our results indicate the potential for an annual and potentially chronic inflow of neonicotinoids into Prairie wetland ecosystems via snowmelt in spring. Clothianidin was detected in 96% of all spring wetland water samples with an additional 43% of those samples also containing thiamethoxam. Over the five week pre-seeding period, regardless of crop or prior treatment, we routinely detected one or more neonicotinoids in all of the wetlands sampled with half of the samples (51%) exceeding the suggested ecological (chronic) threshold of 35 ng/L (Morrissey *et al*., 2015) - in some cases up to six times this safety threshold. Temporary and seasonal wetlands provide critical habitat and food resources for many organisms, especially

during the early breeding season, as these wetlands typically warm and thaw earlier than deeper wetlands providing an initial pulse of production (Hayashi *et al*., 2003). In comparison to imidacloprid, relatively little is known about the field-relevant effects of either clothianidin or thiamethoxam on aquatic insect species (Anderson *et al*., 2015), and even less is known about their chronic toxicity (Morrissey *et al*., 2015).

Previous research has suggested that annual declines in insectivorous farmland birds is linked to depletion of insect food resources that have a larval stage in water (e.g., Diptera, Ephemeroptera, Odonata) with nominal imidacloprid concentrations above 20 ng/L (Hallmann *et al*., 2014). It is unclear if our reported spring neonicotinoid concentrations could also substantially affect food resources in wetlands utilized by songbird, waterfowl or water bird populations. Temporary, seasonal and semi-permanent wetlands account for 20, 44 and 24% of wetland classes utilized by waterfowl during the breeding season, with laying females ingesting up to 49% of their diet in the form of aquatic animal foods (Swanson *et al*., 1985). In our study, all temporary, seasonal, and semi-permanent wetlands contained detectable neonicotinoid concentrations, such that we estimate that up to 88% of waterfowl breeding wetland habitats in agricultural areas could have neonicotinoid contamination.

3.6 Conclusions

Prairie wetlands provide valuable ecosystem services on an annual basis and their importance to aquatic and terrestrial biodiversity is well documented (Bolen *et al*., 1989; Murkin *et al*., 1997; Wrubleski and Ross, 2011). In fact, small wetlands (≥0.2 ha to 4 ha) have been shown to be critical for maintenance of both regional biodiversity and metapopulations of numerous wetland-dependent organisms such as amphibians and small birds (Gibbs, 1993; Semlitsch and Bodie, 1998). Current crop rotation schedules in Canada often include a neonicotinoid-treated seed (e.g., canola) at least every second or third year of planting. In light of these findings of long-term persistence and efficient transport by snowmelt in northern latitude environments, we suggest regulation and mitigation efforts need to further consider the environmental persistence and impact of neonicotinoid insecticides even before spring planting.

3.7 Acknowledgements

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PREFACE TO CHAPTER 4

Neonicotinoid peak concentrations in wetlands and other surface water systems are typically found during the height of the growing season in the early summer months (mid-June to July). In the Canadian Prairies, wetlands often appear to be both ecologically and physically similar, yet my previous research found that the number of neonicotinoid detections and concentrations could vary widely between ponds. Although biotic and abiotic wetland characteristics likely affect environmental persistence of neonicotinoids, the aquatic fate of these insecticides remains unknown. In Chapter 4, my objective was to determine the wetland and landscape features which may be most useful to mitigate neonicotinoid contamination.

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*Minimal changes to the original published manuscript are reflected in the subsequent text including some clarification of overall text and formatting of the references.

CHAPTER 4: ECOLOGICAL AND LANDSCAPE DRIVERS OF NEONICOTINOID INSECTICIDE DETECTIONS AND CONCENTRATIONS IN CANADA'S PRAIRIE WETLANDS

4.1 Abstract

Neonicotinoids are commonly-used seed treatments on Canada's major prairie crops. Transported via surface-runoff into wetlands, their ultimate aquatic fate remains largely unknown. Biotic and abiotic wetland characteristics likely affect neonicotinoid presence and environmental persistence, but concentrations vary widely between wetlands that appear similar ecologically (e.g., plant composition) and physically (e.g., depth) for reasons that remain unclear. We conducted intensive surveys of 238 wetlands, and documented 59 wetland (e.g., dominant plant species) and landscape (e.g., surrounding crop) characteristics as part of a novel rapid wetland assessment system. We used boosted regression tree (BRT) analysis to predict both probability of neonicotinoid analytical detection and concentration. BRT models effectively predicted the deviance in neonicotinoid detection (62.4%) and concentration (74.7%). Detection was best explained by shallow marsh plant species identity (34.8%) and surrounding crop (13.9%). Neonicotinoid concentration was best explained by shallow marsh plant species identity (14.9%) and wetland depth (14.2%). Our research revealed that plant composition, not density, is a key driver of neonicotinoid presence and concentration in Prairie Potholes. We recommend wetland buffers consisting of diverse native vegetation be retained or restored to minimize neonicotinoid transport and retention in wetlands, thereby limiting their potential effects on wetland-dependent organisms.

Key words: fate, landscape ecotoxicology, neonicotinoids, persistence, Prairie Potholes, clothianidin, thiamethoxam, wetland buffers

4.2 Introduction

Global pesticide use is forecast to more than double by the year 2050, which, together with agricultural intensification, could lead to unprecedented losses in both ecosystem services and species richness (Tilman *et al*., 2001). Agricultural intensification relies heavily on pesticide use in which prophylactic and repeated applications across large spatial scales may create the potential for long-term effects at the landscape level (Cairns and Niederlehner, 1996). Between 1

to 10% of pesticides are transported from croplands to wetlands, with even greater losses if rainfall events occur shortly after application (Wauchope, 1978; Fawcett *et al.*, 1994). Frequent and widespread insecticide applications allow for build-up of high concentrations in natural aquatic systems, including wetlands (Main *et al*., 2014). Wetland contamination affects non-target organisms such as aquatic insects and their vertebrate predators (e.g., amphibians, birds) on local and landscape scales. Therefore, to understand the distribution of pesticides in the environment and their effects on biological communities, it is important to conduct locally-intensive surveys across the scales at which pesticides are found (Johnson, 2002).

Pesticide concentrations in the wetland environment can be highly variable (Donald *et al*., 1999; Donald *et al*., 2001; Budd *et al*., 2009; Anderson *et al*., 2013) as their fate is likely influenced by the interplay of numerous physical, chemical and biological variables. Physico-chemical properties of pesticides such as water solubility, molecular weight, soil adsorption and half-life are well described (Gavrilescu, 2005; Arias-Estévez *et al*., 2008). However, environmental persistence of pesticides in surface water is influenced by numerous processes which include: transport, leaching, transfer between environmental compartments (e.g., air, water, sediment) and transformation processes that can change or degrade the compound (e.g., photolysis) (Goldsborough and Crumpton, 1998; Sarmah *et al*., 2004; Gavrilescu, 2005; Rice *et al*., 2007; Arias-Estévez *et al*., 2008). Moreover, both biotic and abiotic environmental factors as well as landscape characteristics can significantly affect the strength persistent contaminants have on biological communities (Beketov and Liess, 2012). Few studies have examined features which may influence detection and concentration of pesticides in aquatic systems such as wetlands.

This knowledge gap is especially true of neonicotinoid insecticides, which are extensively used across agro-wetland landscapes (e.g., Canadian Prairies, Great Plains and Midwestern, United States; Anderson *et al*., 2013; Hladik *et al*., 2014; Main *et al*., 2014; Smalling *et al*., 2015). Neonicotinoids are frequently applied in spring as soil or seed treatments or in summer as a foliar spray. As seed treatments, they act as a systemic which protects the roots and shoots of young plants against piercing-sucking insects such as aphids, thrips and some Coleopteran pests (e.g., wireworm) (Jeschke *et al*., 2010; Goulson, 2013). Together, the three most commonly-used neonicotinoids - thiamethoxam, clothianidin, and imidacloprid - are

registered for use in 120 countries on 295 crops, including canola, cereals, corn, rice, vegetables and soybeans (Elbert *et al*., 2008). Application rates vary between crops and neonicotinoid active ingredient (e.g., corn – imidacloprid, 11-96 g a.i./ha; wheat – thiamethoxam, 36-63 g a.i./ha) (Douglas and Tooker, 2015).

Abiotic factors such as surrounding soil or topography (e.g., organic matter, slope), water (e.g., depth and temperature), dissolved organic carbon and admittance of solar ultraviolet light may play a role in neonicotinoid fate and persistence in surface waters (Peña *et al*., 2011). Biotic factors such as submerged or emergent plant growth could provide opportunities for surface adsorption, chemical sequestration in plant tissues or even increased exposure to solar irradiance for photolysis (Goldsborough and Crumpton, 1998). Numerous other factors such as depth, pH, turbidity, and surficial cover through algal bloom formation are speculated to affect neonicotinoid persistence in surface waters (Morrissey *et al*., 2015). Field margin vegetation and other plants may draw up neonicotinoids from arable soils (Goulson, 2013). Persistence of systemic pesticides in aquatic environments will necessarily vary with field conditions (Bonmatin *et al*., 2015). There is an immediate need to better understand the fate of neonicotinoids in the aquatic environment – especially the less studied clothianidin and thiamethoxam (US EPA, 2010; Anderson *et al*., 2015) - as these and other neonicotinoids will impact ecosystem services and functions (Chagnon *et al*., 2014) with potential consequences for wetland environments and communities.

In this paper, neonicotinoids are presented as a case study through examination and modelling of the numerous variables that may affect pesticide detections and concentrations in wetlands. Previous wetland studies, although limited, have found neonicotinoids to be prevalent in the environment (Anderson *et al*., 2013; Main *et al*., 2014); however, a range of neonicotinoid concentrations have been consistently detected across these and other surface water systems worldwide (Morrissey *et al*., 2015). Main *et al*. (2014) found little accumulation of neonicotinoids in wetland sediments; however, neonicotinoids were shown to both accumulate (Bonmatin *et al*., 2005a) and persist in agricultural soils for extensive periods of time (US EPA, 2010; Goulson, 2013; Jones *et al*., 2014). Higher latitudes will experience longer half-lives due to lower light intensity as well as lower annual temperatures overall (Bonmatin *et al*., 2015). As wetlands are the transition zone between aquatic and terrestrial ecosystems, they may be at

greater risk for potential pesticide contamination. Neonicotinoids are highly water soluble and have low soil adsorption and lipophilicity which increases the likelihood of their movement into aquatic systems via surface and sub-surface runoff (CCME, 2007; HSDB, 2012).

Here, we present an exploratory analysis demonstrating the potential for modelling both neonicotinoid detection and concentration in a large number of wetlands in Canada's Prairie Pothole Region using a set of 59 biotic and abiotic variables collected as part of a field-validated rapid wetland assessment system. We hypothesized that landscape features such as crop type and topography, and wetland features such as surficial or surrounding plant cover could predict neonicotinoid detections and concentrations. Ultimately, our objective was to determine those features which may be most useful to mitigate neonicotinoid contamination to aid management and conservation decisions regarding wetland ecosystems.

4.3 Methods

4.3.1 Study sites and wetland selection

Fieldwork was conducted in 2012 and 2013 on 238 wetlands embedded within intensive agricultural cropland or grass/hay fields located near 10 rural communities across central Saskatchewan (Fig. 4.1). Agricultural fields were selected using a stratified random design to encompass a wide range of land use and crop types, including: barley, canola, chemfallow (herbicide-treated fallow fields), flax, field pea, grassland (including pasture and prairie), oat and wheat. We sampled wetlands of varying size and permanency (=class). Where possible, we sampled three wetland replicates per agricultural quarter section (= 65 ha field; Main *et al*., 2014) across a range of wetland classes (temporary, seasonal, semi-permanent and permanent) (Stewart and Kantrud, 1971). Prairie Pothole wetlands are often highly disturbed due to surrounding land use activities (e.g., crop production). Consequently, vegetation communities are commonly dominated by disturbance-tolerant species such as broadleaf cattails (*Typha latifolia*). Since the wetland hydrology of the Prairie Pothole Region is reliant upon the accumulation of snowmelt during spring runoff, the size, depth, vegetation communities, and range of available wetland class often differed among the 10 sampling areas based on local-scale variation in snowfall (Conly and van der Kamp, 2001; van der Kamp and Hayashi, 2009).

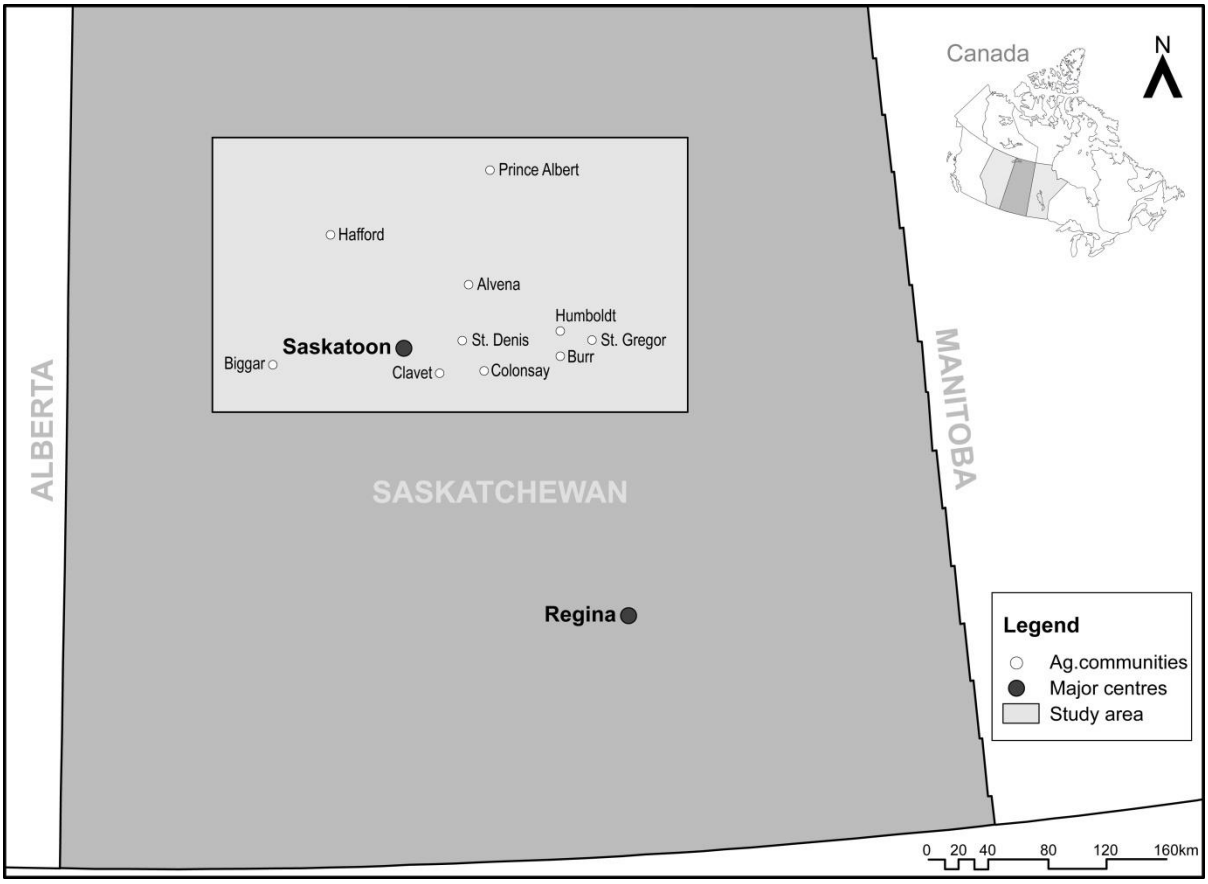

Figure 4.1 Map of wetland study area and nearest rural agricultural community locations throughout central-Saskatchewan.

4.3.2 Water sampling

A single water sample was collected during a 10 day period (late-June to early July) to capture effects of the agricultural growing season and growth and diversity of wetland vegetation, but not necessarily peak pesticide concentrations. A detailed description of the water sampling methods used in this study is outlined in Main *et al* (2014).

4.3.3 Rapid wetland assessment criteria

Our rapid wetland assessment method was modified from published Prairie Pothole Region classification systems (Stewart and Kantrud, 1971; Millar, 1976), rapid assessment criteria (Spencer *et al*., 1998; Fennessy *et al*., 2007; Kotze *et al*., 2012) and vegetation evaluation techniques (Kantrud and Newton, 1996; Guntenspergen *et al*., 2002; DeKeyser *et al*., 2003; DeKeyser *et al*., 2009) that included both quantitative and qualitative indicators. Field-validated

criteria were based on 59 environmental predictor variables grouped into five categories (see Appendix Table A1 for full list of variables measured). Wetland water depth was measured in the central portion of the pond and width of vegetation zonation was recorded at the four major compass points of each wetland (Spencer *et al*., 1998). Vegetation in Prairie Pothole wetlands typically exhibit classic patterns of zonation with individual species distribution along a moisture or water depth gradient (Guntenspergen *et al*., 2002). Classification of plant zones was chosen based on the majority of species that fall within categories from outer to inner zones – wet meadow, shallow marsh, emergent deep marsh and shallow open water (Stewart and Kantrud, 1971; Millar, 1976). The boundary of study wetlands was based on the edge extent of the wet meadow zone, i.e., areas that contain water for several weeks after snowmelt. We only included plant communities equaling >10% of the plant zone area, thus rare plant species were not included (Kantrud and Newton, 1996). Wetland coordinates and area calculations of all wetlands were completed using a hand-held GPS unit (Garmin Montana 650). Visual estimations of variables such as percent cover of surficial vegetation or vegetation disturbance levels were obtained by the same surveyors in both years to reduce observer error or bias. Landowner rotation schedules and/or plant identification were used to identify surrounding crops. Neonicotinoid application rates were not available for individual fields. Instead, surrounding crop was used as a proxy given the known frequency and standard rates of application (Main *et al*., 2014).

4.3.4 Neonicotinoid analysis: water samples

Wetland water samples were analyzed at the National Hydrology Research Centre, Environment Canada in Saskatoon, SK. Methods described herein were previously adapted from Xie *et al* (2011). Analytical standards of acetamiprid, clothianidin, imidacloprid and thiamethoxam were purchased from Chem Service (West Chester, PA, USA) and the internal standard, d_4-imidacloprid, was purchased from CDN Isotopes (Pointe-Claire, QC, Canada). A detailed description of the sample extraction and LC/MS/MS analysis is outlined in Main *et al*. (2014). Limits of quantification (LOQ) in wetland water were: thiamethoxam, 1.6 ng/L; imidacloprid, 1.1 ng/L; clothianidin, 0.9 ng/L and acetamiprid, 0.7 ng/L. Mean recoveries (n=9) from wetland water fortified at 50 ng/L were as follows: acetamiprid, 90.4±4.13%; imidacloprid, 88.9±4.79%; thiamethoxam, 87.1±5.50% and clothianidin, 83.8±7.33%. All neonicotinoid

concentrations were recovery corrected between batches and laboratory and field blanks were all below detection. As 34% of our wetlands contained >2 neonicotinoid mixtures, we used the sum of total neonicotinoid concentrations per wetland as the overall response variable in our models. We treated samples with neonicotinoid concentrations all below the limit of quantification (LOQ) as non-detections (zeroes).

4.3.5 Boosted regression tree (BRT) models

Boosted regression trees (BRTs) are a machine-learning method used in ecology and conservation biology for analysis and modelling, particularly in exploratory contexts (De'Ath, 2007; Elith *et al*., 2008; Illán *et al*., 2014; Soykan *et al*., 2014). We used BRTs to examine the relative influence of 59 different biotic and abiotic wetland and landscape predictors on neonicotinoid analytical detections >LOQ (hereafter detections) and total neonicotinoid concentrations. Classification and regression trees are ideal for ecological modelling because they accommodate complex linear and nonlinear responses to multiple categorical and continuous predictors, are relatively insensitive to collinearity and outliers, allow complex interactions, can accommodate missing data, and allow the model to be developed from the data rather than predetermined (Elith *et al*., 2008). However, single classification or regression trees may not represent the optimal model, can be difficult to interpret, and may produce biologically unrealistic models. BRTs improve upon single classification and regression trees by incorporating "boosting," in which a large number of trees are built and combined in an iterative process to improve model performance (Elith *et al*., 2008; Illán *et al*., 2014).

BRT models require the specification of three parameters: learning rate, tree complexity and bag fraction (Elith *et al*., 2008). The learning rate determines the contribution of each tree to the growing model and how quickly the model will converge on a solution. Slower learning rates produce better predictive capacity of the overall model, but increase processing time. Learning rates within the range of 0.001 – 0.1 are recommended (Elith *et al*., 2008; Soykan *et al*., 2014). Tree complexity - the number of nodes in an individual tree – determines the maximum number of interactions between the predictor variables. Bag fraction specifies the proportion of the training data that is used for model building at each step (Elith *et al*., 2008).

We use an iterative tuning process to select our model parameters (learning rate, bag fraction, and tree complexity; per Elith *et al*. 2008). We started with an intermediate learning rate of 0.01 and iteratively evaluated faster (=larger) or slower (=smaller) learning rates until we reached an optimum trade-off between mean deviance explained and number of trees produced (~1,000-2,000) (Elith *et al*., 2008). We obtained a learning rate of 0.002 for the neonicotinoid detection model and 0.003 for the concentration model. Similarly, we iteratively evaluated bag fractions within the recommended range of 0.5-0.75 and selected the value that explained the most model deviance. We assigned a fixed tree complexity of 3 (per Soykan *et al*. 2014), as this allows two- and three-way interactions between predictor variables. Higher-order interactions are too complex to identify within our dataset, and are unnecessary to explain additional variation in neonicotinoid distribution.

The data were strongly zero-inflated due to the sampling of many ponds with total neonicotinoid concentrations <LOQ. As a result the data yielded a poor fit to the theoretical distributions available within the BRT framework: Gaussian, Poisson, and binomial. Therefore we used a two-step modelling process based on the delta-lognormal approach commonly used to analyze zero-inflated count data (Lo *et al*., 1992; Abeare, 2009). We first collapsed the response variable to produce a binary dataset, in which 0 indicates a concentration <LOQ (=analytical non-detection) and 1 indicates neonicotinoid concentrations >LOQ (=analytical detection). We also produced a zero-truncated dataset in which all values <LOQ were removed and concentrations were retained. The response values in the concentration dataset were first log-transformed to produce normally-distributed errors (Elith *et al*., 2008). We ran two BRT models on these datasets to separately identify the factors that influenced analytical detection and concentration of total neonicotinoids in Prairie wetlands. We assessed collinearity by calculating pairwise Pearson's correlation coefficients between continuous predictor variables. Although collinearity does not affect BRT predictions, it can influence VI scores and partial dependence plots (Soykan *et al*., 2014). Collinearity among predictor variables was low. Only three pairs of predictors had correlation coefficients with absolute values >0.7 (Dormann *et al*., 2013), they measured different things and accounted for less than 7% of the total variance in analytical detection and were not retained in the concentration model.

We evaluated model performance two ways: internal cross-validation of mean percent deviance explained, and the cross-validated mean correlation coefficient between our model predictions and observed data (Elith *et al*., 2008; Soykan *et al*., 2014). We were unable to externally cross-validate our model due to our limited sample size relative to the large number of environmental predictor variables. Internal cross-validation partitions the data into training and validation data sets that are used to assess the predictive power of the model on an independent data set, while allowing the complete data set to be used in model development. The mean correlation coefficient (*r*) between model predictions and observed data is a useful index of the similarity between model output and reality (Soykan *et al*., 2014).

The relative influence of wetland and landscape predictor variables are presented as variable importance (VI) scores. VI scores are calculated based on the number of times the variable occurs in the set of trees, weighted by how much the variable improves the fit of each tree, averaged across the entire model. Variables with higher VI scores have a stronger influence on the response (neonicotinoid detection or concentration); all VI scores sum to 100 (Elith *et al*., 2008; Soykan *et al*., 2014). Because these VI scores are relative and do not indicate whether given variables are useful for modelling a response, we added a random number between 1 and 100 as an additional predictor variable (Soykan *et al*., 2014). Including a random number allows the identification of variables that have stronger influences on neonicotinoid detection and concentration than random. We retained predictor variables with higher VI scores than the random number in the final models. All analyses were conducted in package *dismo* (Hijmans *et al*., 2012) in R version 3.0.1 (R Development Core Team 2014).

4.4 Results

4.4.1 Neonicotinoid concentrations in wetland water

We sampled water from 134 wetlands in June of 2012 and 145 wetlands in June of 2013. Only those wetlands still holding water in early July were included in our rapid wetland assessments: (2012: 126 wetlands and 2013: 112 wetlands). In 2012, 83 of 134 wetlands (62%) contained at least one neonicotinoid versus 123 of 145 wetlands (85%) in 2013 (see Main *et al*., 2014; Table 4.1). All four neonicotinoids assessed were detected in at least one sample. Detection frequency was ranked in both years as follows: clothianidin > thiamethoxam >

imidacloprid > acetamiprid. Mean and maximum concentrations by active ingredient varied in both years (Table 4.1) and thus sampling year was included in BRT models. Mean and maximum total neonicotinoid concentrations decreased with wetland permanence as follows: temporary (mean: 272.4 ng/L; max: 590); seasonal (mean: 198.6 ng/L; max: 3110); semi-permanent (mean: 96.8 ng/L; max: 520.4); and permanent (mean: 22.6 ng/L; max: 129.3).

Table 4.1 Summary of detections, arithmetic means and maximum concentrations of total (summed) neonicotinoids and active ingredients in water from Prairie wetlands of central Saskatchewan (June to July, 2012-2013). ng/L = nanograms per liter.

Sampling Period	Neonicotinoid	% Detection	Mean (ng/L)	Max. (ng/L)
Summer 2012	Clothianidin	51	142	3110
n = 134	Thiamethoxam	19	40.3	1490
	Imidacloprid	8.2	15.9	256
	Acetamiprid	1.5	1.1	54.4
	Total Neonic.	**62**	**76.8**	**3110**
Summer 2013	Clothianidin	76	59.7	498
n = 145	Thiamethoxam	52	40.6	476
	Imidacloprid	12	7.1	196
	Acetamiprid	0.69	0.6	13.4
	Total Neonic.	**85**	**108**	**595**

4.4.2 BRT model performance

The BRT models accounted for 62.4% of the deviance in neonicotinoid detections and 74.7% of the deviance in total neonicotinoid concentrations (Appendix A, Table A2). Neonicotinoid detections were explained by 21 variables, including the top four which explained the majority of variance: dominant shallow marsh plant species identity (importance value = 34.8, indicating that it explained 34.8% of model deviance); current crop type (13.9%); vegetation disturbance (13.0%) and dominant wet meadow plant species identity (5.0%; Fig. 4.2A).

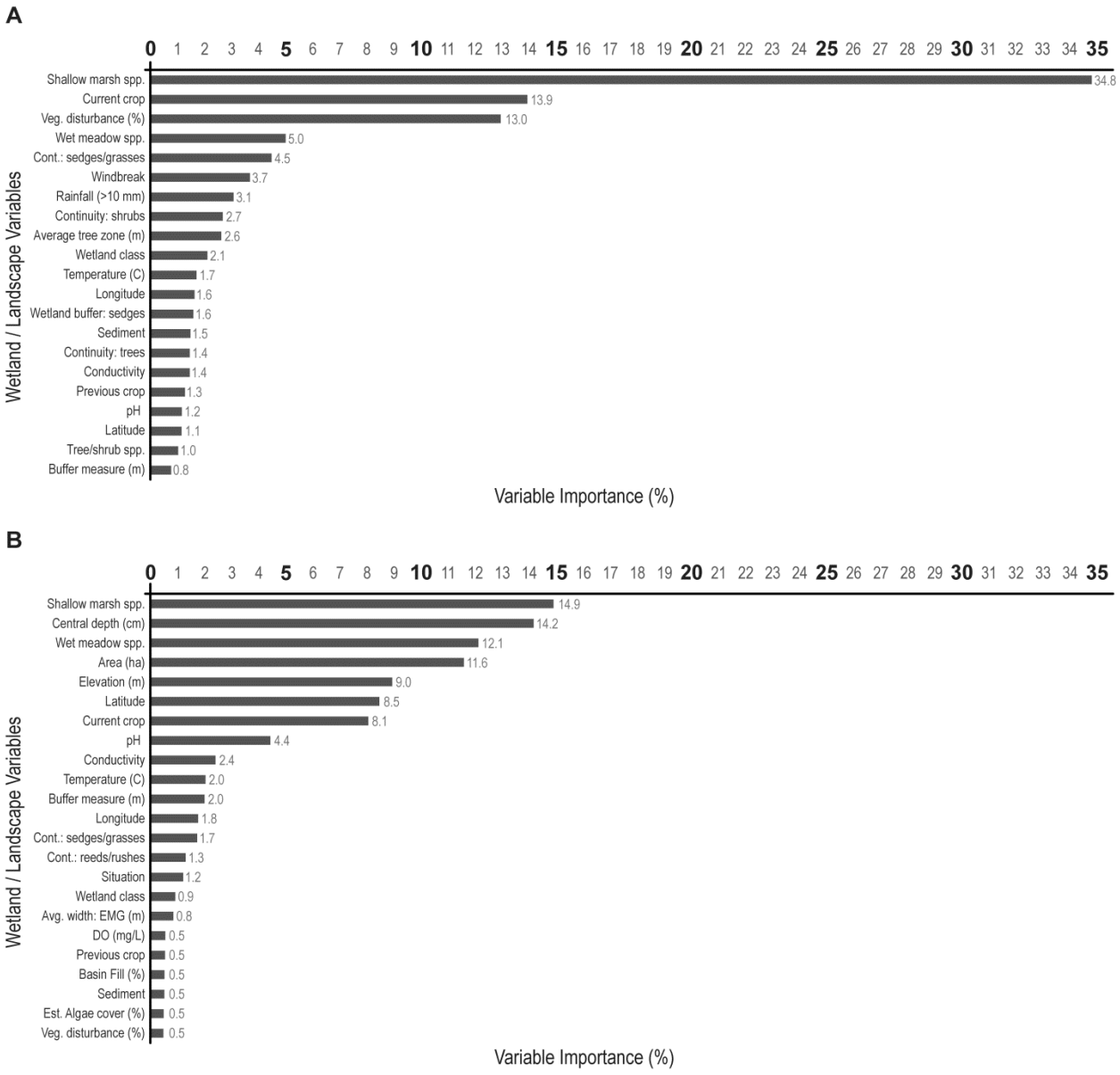

Figure 4.2 Variable importance (VI) scores of key wetland and landscape predictor variables affecting neonicotinoid frequency of detection (A) and total (summed) concentration (B) in Prairie wetlands. The sum of scores is equal to 100 with percentages of predictor variables indicating the importance of that variable contributing to the overall deviance of the model.

Within the shallow marsh zone, the dominant plant species identity (n = 28) was associated with neonicotinoid detection probability. A high neonicotinoid detection probability (marginal effect ≥ 1.0) was predicted by the presence of four dominant plant species: *Thlaspi*

arvense, *Agrostis scabra*, *Poaceae spp*. and *Avena fatua*. A low detection probability (marginal effect ≤ 1.0) was predicted by the presence of four dominant plant species: *Aster borealis*, *Sium suave*, *Carex bebbii* and *C. atherodes* (Fig. 4.3A). Wetlands surrounded by crops including oat, canola and barley had a higher detection probability whereas neonicotinoids were rarely detected in wetlands within pasture and grasslands (Fig. 4.3B). Neonicotinoid detections increased nonlinearly with vegetation disturbance with two thresholds (sharp increases) at approximately 45% and 80% disturbance levels (Fig. 4.3C). Dominant wet meadow species also influenced detections. Fourteen dominant plant species were associated with a higher probability of neonicotinoid detection (marginal effect >0), including *Plantago major*, *Juncus torreyi*, and *A. fatua*, while seven species, including *Scolochloa festucacea* and *Equisetum hyemale* were associated with a lower detection probability (Fig. 4.3D).

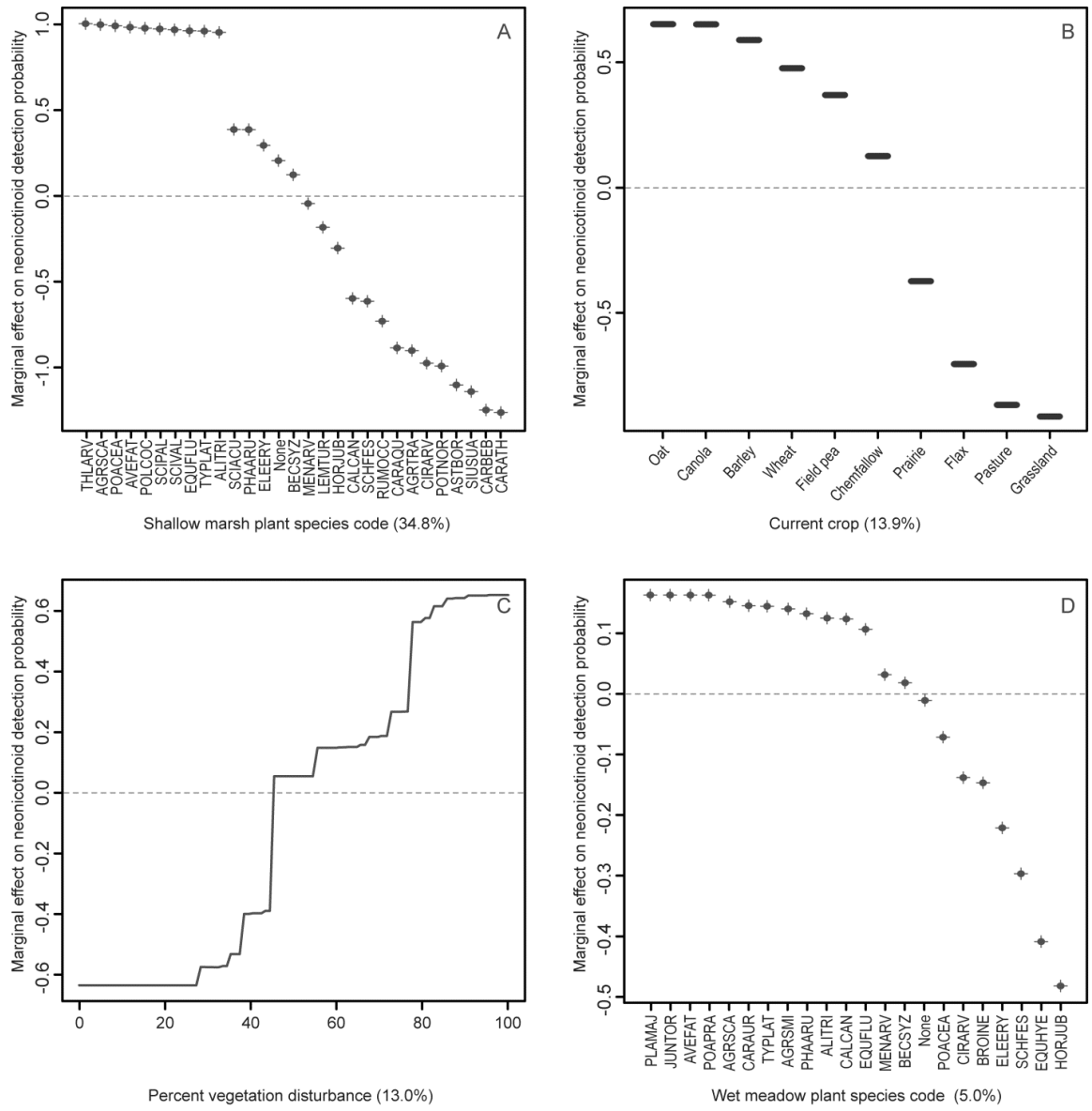

Figure 4.3 Relationships between frequency of detection of total (summed) neonicotinoids in Prairie wetlands and key predictor variables: (A) shallow marsh plant species, (B) current crop, (C) percent vegetation disturbance, (D) wet meadow plant species. Plots are presented as dimensionless transformations of the response variables with variable importance (VI) scores listed for each variable. Plant species codes are defined in Appendix A, Table A3.

Neonicotinoid concentrations were explained by 23 variables, including the top seven which explained the majority of variance: dominant shallow marsh plant species (14.9%), wetland central depth (14.2%), dominant wet meadow plant species (12.1%), area (11.6%), elevation (9.0%), latitude (8.5%) and current crop (8.1%; Fig. 4.2B). We found higher neonicotinoid concentrations (marginal effect >0), were predicted by dominant shallow marsh plant species identity (n = 11) including *T. arvense*, *Scirpus validus*, and *Carex aquatilis*. Lower neonicotinoid concentrations were predicted by ten species including *Scirpus acutus* and *Mentha arvensis* (Fig. 4.4A). Wetland central depth had a negative nonlinear effect on neonicotinoid concentration, with sharp concentration declines at depths of 25 and 40 cm (Fig. 4.4B). Neonicotinoid concentrations were also predicted by wet meadow dominant plant species identity (n = 18), with the presence of six species associated with higher neonicotinoid concentrations (marginal effect >0.2), including: *A. fatua*, *Calamagrostis canadensis* and *E. fluviatile* (Fig. 4.4C). Wetlands which were missing wet meadow plant communities (= "none") were also associated with higher neonicotinoid concentrations. Wetlands smaller than ~1ha in size (Fig. 4.4D) and those wetlands situated at lower elevations (450m to ~525m) were more likely to have higher total neonicotinoid concentrations, whereas concentrations decreased sharply at elevations above 550m (Appendix A, Fig. A1). Concentrations exhibited a regional pattern in which they peaked at latitudes near 52.6ºN (Appendix A, Fig. A2). Consistent with neonicotinoid seed treatment use and rotation patterns, wetlands surrounded by the crops of canola, oat and barley had higher concentrations than wetlands surrounded by wheat and field pea (Appendix A, Fig. A3).

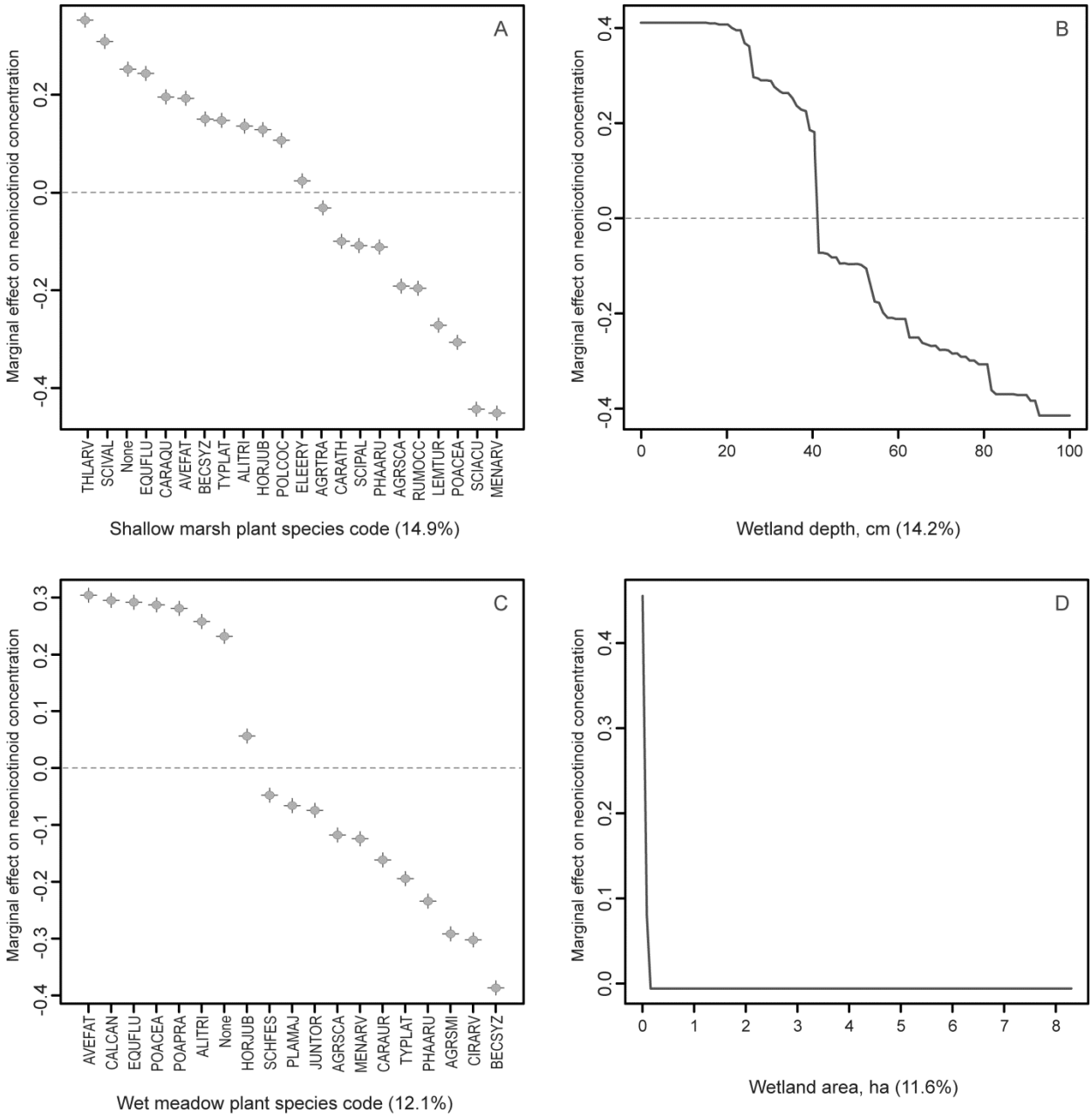

Figure 4.4 Relationships between concentration of total (summed) neonicotinoids in Prairie wetlands and key predictor variables: (A) shallow marsh plant species, (B) wetland depth, (C) wet meadow plant species, (D) wetland area. Details are the same as listed in Figure 2. Plant species codes are defined in Appendix Table A3.

4.5 Discussion

Here we present a modelling approach that effectively explains neonicotinoid detections and concentrations in Prairie wetlands based on locally-collected and remotely-sensed environmental variables. To our knowledge, we are the first to simultaneously assess the relative

effects of multiple vegetative and biogeophysical characteristics on neonicotinoid fate in wetlands across multiple scales. Three variables, dominant shallow marsh plant species, crop type and percent vegetation disturbance, accounted for over half (62%) of the variation in neonicotinoid detection probability; three variables (dominant shallow marsh and wet meadow plant species and wetland depth) also accounted for over 40% of the variation in neonicotinoid concentration.

Of the 59 variables included in our model set, vegetation community structure in the shallow marsh zone best explained both neonicotinoid detections (34.8%) and concentrations (14.9%). However, shallow marsh species presence was a more important predictor of detections than concentrations as the greater species richness in the detection model equated to more variation explained. The shallow marsh zone is often described as a "buffer zone" for wetlands and, in the case of neonicotinoids, has an overwhelming effect on the transport and persistence of this pesticide class. Detection of neonicotinoids was highest in wetlands containing select dominant plant species in the shallow marsh, including: *T. arvense*, *Scirpus paludosus*, *T. latifolia* and *Alisma triviale* (Appendix Table, A3). All four species have some ability to tolerate disturbance. *T. arvense* is resistant to soil disturbance; *S. paludosus* and *T. latifolia* can be pioneering species after disturbance often withstanding even slightly brackish conditions; and *A. triviale* is typically prevalent in wetlands adjacent to cultivated fields (Stewart and Kantrud, 1972). Conversely, wetlands dominated by sedge species such as *C. atherodes* were likely to be devoid of neonicotinoids. Similar to the detection model, neonicotinoid concentrations were highest in wetlands containing species such as *T. arvense*, *Carex aquatilis*, and *S. validus*. The lowest neonicotinoid concentrations were detected in wetlands where *M. arvensis* or *S. acutus* dominated plant communities in the shallow marsh.

It is unclear as to why certain plant species are associated with higher or lower neonicotinoid detections or concentrations in these wetlands; however, wetlands and their associated vegetation have been documented to increase the removal of a range of pesticides through plant uptake, root sorption, and hydrolysis (Gregoire *et al*., 2009; Maillard *et al*., 2011; Vymazal and Březinová, 2015). We speculate that some of these dominant species may also have phytoremediation potential or may alter their abiotic environment leading to increased/decreased persistence. In later wetland hydroperiod stages, plants such as *A. triviale* and *P. arundinacea*

have greater surface area (e.g., roots, shoots and leaves) within the water, which may increase sorption to plants and create biofilms that promote microbial activity and pesticide degradation (Elsaesser *et al*., 2011). The presence of specific dominant plant species may also be representative of geophysical characteristics of certain study wetlands including landscape position, water depth, and water quality. Although we measured each of these variables separately and the model ranks them with VI scores, we cannot rule out the possibility that plant species identity could act as a surrogate for the complex ecological interactions between numerous individual variables.

We also cannot rule out the possibility that the causality of the association between neonicotinoid detection and concentration and plant species composition is reversed. Neonicotinoids may be acting as a stressor that affects some plant species more than others, leading to altered plant community compositions. For example, *T. arvense* – a member of the Brassicaceae family – is indicative of wetlands with both high concentration and high probability of neonicotinoid detection. A non-native and noxious weed, *T. arvense,* is likely tolerant to a variety of stressors, potentially including neonicotinoids, as it is known to be resistant to soil disturbance and some herbicides (Beckie *et al*., 2007). Further experimental study of the relationship between neonicotinoids and individual and community plant responses is needed.

Aquatic plants also provide physical structure to wetlands and are able to alter the wetland's abiotic environment both physically (e.g., light levels) and chemically (e.g., pH, nutrient levels; van der Valk, 2012). This feature could influence neonicotinoid fate and persistence in Prairie wetlands. However, our findings reveal that plant species composition, more so than density, affects neonicotinoid detections and concentrations. We assessed plant density in two ways: 1) actual measurements of vegetation zones (e.g., width); and 2) cover type, which identifies the ratio of plants to open water (Stewart and Kantrud, 1971; Millar, 1976). Cover type was not retained in our final models and less than 2% of model variance was accounted for by actual vegetation zone measurements.

Presence of intact wet meadow and shallow marsh zones is an important indicator of healthy wetland condition (Guntenspergen *et al*., 2002). Although detection of neonicotinoids was strongly influenced by certain dominant plant species in the shallow marsh and wet meadow zones, high total neonicotinoid concentrations were frequently found in wetlands where those

zones had been removed or cultivated. The sharpest increases in neonicotinoid detection probability were found at 45% vegetation disturbance and ~80% disturbance. This factor was not important to our concentration model possibly indicating a trade-off between disturbance, which facilitates chemical transport, while also enhancing dilution from runoff. Due to their rich, moisture-retaining soils, wet meadow and shallow marsh zones tend to be cultivated during dry years which create coarse-grained vegetation mosaics with fewer native plants (Kantrud and Newton, 1996). In our study, 62% of 238 wetlands were missing an outer wet meadow community. Removal of these buffer vegetation zones likely increased their susceptibility to neonicotinoid contamination from adjacent cultivated row crops. Presence of wet meadow grasses may trap and degrade various pesticide compounds (Vianello *et al*., 2005). Further research has shown that plant buffer strips or vegetated treatment systems are the most important characteristic influencing the retention performance of pesticides leading to degradation via hydrolysis or photolysis (Stehle *et al*., 2011). The presence or absence of buffer zones has important implications for protection of aquatic organisms such as wetland macroinvertebrates. Buffered wetland sites are known to hold significantly greater aquatic macroinvertebrate abundance than non-buffered wetlands (Riens *et al*., 2013).

Our model indicated that depth accounted for 14.2% of variation in neonicotinoid concentration, with the highest concentrations in shallow wetlands <40cm deep. Wetland size and depth are frequently correlated, such that many of these shallow high-concentration wetlands were <1 ha in size. Indeed, wetland area accounted for 11.6% of variation in neonicotinoid concentration. The highest concentration detected in our study (3,110 ng/L; Main *et al*., 2014) was in a seasonal wetland of 0.3 ha in size and 12 cm depth; whereas, our largest study wetland (83 ha and >1m deep) had a concentration of 5.8 ng/L. Although orders of magnitude lower, likely due to dilution, many of these larger more permanent waterbodies contain detectable levels of neonicotinoids, indicating movement into water bodies of all sizes. Deeper wetlands frequently have less sunlight penetration which may slow neonicotinoid degradation, facilitating persistence, despite low concentrations. Indeed, thiamethoxam underwent a slower degradation process in the absence of light (Peña *et al*., 2011). Our finding of high concentrations in shallow wetlands seemingly contradicts the prediction of rapid neonicotinoid degradation in shallow waters due to extensive light penetration and photolytic breakdown (Goldsborough and Crumpton, 1998). However, many Prairie wetlands are highly turbid, contain a high variability

of dissolved organic carbons, or are heavily disturbed which likely reduces light penetration and, consequently, degradation. Neonicotinoid persistence in natural environments could potentially be influenced by dissolved organic matter acting as a photosensitizer (Zeng and Arnold, 2012).

Crop type is known to influence neonicotinoid concentration in Prairie Pothole wetlands (Main *et al*., 2014). Current crop type accounted for 13.9% of variation in neonicotinoid detection and 8.1% of variation in concentration. Over 95% of canola grown in Canada is treated with a neonicotinoid active ingredient (Main *et al*., 2014). Although registered for use, most Canadian cereal (e.g., oat, barley) and legume (e.g., field pea) crops are less frequently treated with neonicotinoids; however, treated-seed options continue to grow in spatial extent and frequency of use. Wetlands in untreated grassland (including prairie and pasture) and chemfallow fields still contain trace neonicotinoids likely from previous crop treatments (Goulson, 2013; Jones *et al*., 2014).

Year effects including time since planting and rainfall may also influence concentrations of neonicotinoids within wetland water. We sampled wetlands during the same 10 day time period (late June to early July) in both years. We did not aim to capture peak water concentrations which typically occur within 24 h post-seeding, followed by rapid breakdown via first-order kinetics over the next 48 h and slower degradation over the subsequent 192 h time period (Armbrust and Peeler, 2002; Morrissey *et al*., 2015). In Prairie wetlands, rainfall events can strongly increase the potential for surface water contamination (Chiovarou and Siewicki, 2008). Although rainfall and temperature patterns differed between years, year effects did not explain detection or concentration in either model. We did not specifically target our wetland sampling to include precipitation events, but did include time since last rainfall (defined as a precipitation event ≥10mm) as a variable in our model. Similarly, time since last rainfall had a minimal effect (3.1%), with peak detection probabilities between ~3 and 12 days post-rainfall. Rainfall did not contribute to the neonicotinoid concentration model.

Several biotic variables (e.g., surficial plant and algal cover) and abiotic variables (e.g., pH) had minimal to no effect on neonicotinoid detection and concentration, despite the fact that plant community structure in emergent and submerged aquatic zones can influence water temperature and light diffusion (van der Valk, 2012). We hypothesized that surficial plant cover may be associated with higher detection probability and concentration due to reduced photolysis.

However, % algal cover did not contribute to the detection model and accounted for only 0.5% of the variation in neonicotinoid concentrations. Though clothianidin rapidly degrades through photolysis in the lab, this is less likely to occur in natural systems unless they hold clear, irradiated water (US EPA, 2010). Conversely, algal cover may increase pesticide degradation despite reductions in light levels. Algae decreased the persistence of atrazine and lindane through pesticide sorption or algae-facilitated degradation in a lab environment (Friesen-Pankratz *et al*., 2003), and algal blooms reportedly rapidly degrade pesticides under field conditions (Rose *et al*., 2006). Plant growth generally increases a wetland's overall surface area relative to its water volume which may further increase opportunities for surface adsorption, sequestration in plant tissues, microbial degradation and improved exposure of compounds on leaves to sunlight for photolysis (Goldsborough and Crumpton, 1998; Gregoire *et al*., 2009; Vymazal and Březinová, 2015).

Interestingly, landscape features such as topography and wetland density, although speculated to have an influence on presence and concentration of neonicotinoids, were found to account for minimal to no variance in our models. We found no effect of wetland density on neonicotinoid detection and concentration, contrary to previous studies showing that numerous small "pothole" wetlands interspersed with intensive agriculture have high probabilities of chemical contamination (Grue *et al*., 1986). We used wetland situation (isolated, overflow, channel and terminal) as a topographic proxy measurement of a wetland's position in the landscape or watershed (Millar, 1976). Wetland situation accounted for only 1.2% (concentration) of the overall variance in our models. The highest concentrations in our study were found in isolated wetlands or those receiving water from surrounding uplands, but never overflow (Millar, 1976). Although we anticipated terminal wetlands – found in topographically low areas – to have the highest concentrations, isolated wetlands (245 ±60 ng/L) had higher mean (±SE) concentrations than terminal connected wetlands (53.7 ±7.4 ng/L). Elevation did account for 9.0% of concentration model variance, and lower-elevation (~450 – 550m) wetlands had higher overall neonicotinoid concentrations. Many of our study wetlands appear biologically unique and independent of one another; therefore, wetland density and landscape situation were less important features.

4.6 Implications for wetland ecosystem conservation

Numerous plant species in the shallow marsh and wet meadow zones were indicative of both detection and concentration of neonicotinoids. However, wetlands lacking these two zones had the highest neonicotinoid concentrations, further indicating the importance of preserving healthy vegetation communities in agricultural wetlands. Cultivation of both wet meadow and shallow marsh vegetation communities can increase the potential for chemical inputs such as pesticides (Kantrud and Newton, 1996).

Neonicotinoids are implicated in declines of a variety of animals, most notably bees (Krupke *et al*., 2012; Fischer *et al*., 2014; Samson-Robert *et al*., 2014; Williamson *et al*., 2014). In wetlands and other surface water systems, numerous non-target aquatic insect species may experience direct toxic effects or sublethal effects (e.g., reduced emergence), which inhibits critical ecosystem functions such as leaf litter breakdown and nutrient cycling (Alexander *et al*., 2008; Kreutzweiser *et al*., 2009; Van Dijk *et al*., 2013). Long-term neonicotinoid concentrations in water that exceed 35 ng/L can affect sensitive aquatic invertebrate populations through chronic effects (Morrissey *et al*., 2015). Importantly, over half (52%) of the wetlands in our study surpassed the critical value above which invertebrates are affected. Our research, combined with previous documentation of pervasive use and frequent wetland occurrence of neonicotinoids throughout the Canadian Prairies (Main *et al*., 2014), reveals that agriculturally embedded wetlands and their associated communities are impacted. Pothole wetlands are chronically exposed based on growing neonicotinoid use across the Canadian Prairies (Main *et al*., 2014), with potential for negative effects of aquatic macroinvertebrates and the insectivorous birds, fish, amphibians, and mammals that rely upon them (Gibbons *et al*., 2015).

Part of the objective of wetland ecotoxicology should be to determine management strategies to ameliorate impacts on these chemically-stressed systems (Catallo, 1993). Our study highlights the value of using robust techniques to analyze complex multivariate datasets including a wide range of biotic and abiotic wetland and landscape characteristics. Our findings reveal that neonicotinoid presence and concentration in surface water are largely affected by the presence and composition of vegetated wetland buffers, whereas many abiotic and landscape variables show limited influence. This observation is timely and important for understanding how an extensively used chemical class like neonicotinoids behaves under field conditions.

Temporary and seasonal wetlands are most often disturbed and seem to be under the greatest impact from chemical contamination, despite their importance for supporting biodiversity including invertebrates and their vertebrate predators (Kantrud and Newton, 1996; Guntenspergen *et al.*, 2002). Mechanistic explanations of the patterns described herein are beyond the scope of this exploratory study. Further research, particularly experimental approaches, is encouraged to identify the processes and causality. We recommend research to examine the individual contribution of key wetland habitat variables highlighted here on pesticide fate. Regardless of the mechanism, our research has clear and important implications for management of agricultural landscapes. To reduce neonicotinoid concentrations in wetlands – and their concomitant effects on aquatic insects, and other organisms – buffers consisting of diverse native vegetation should be retained.

4.7 Acknowledgements

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PREFACE TO CHAPTER 5

Width of surrounding vegetation has often been a determining factor in the ability to effectively mitigate pesticide run-off into agricultural wetlands. However, as the results of Chapter 4 demonstrated, buffer width was not nearly as important as the presence of certain shallow marsh plant species in explaining both neonicotinoid detection and concentration in Prairie wetlands. It is unclear if certain plants have the ability to phytoremediate or filter neonicotinoid insecticide inputs from surrounding agricultural lands. To that end, the objective of this chapter was to examine the ability of common wetland macrophytes to uptake and/or mitigate neonicotinoid residues entering surface waters.

CHAPTER 5: THE ABILITY OF WETLAND MACROPHYTES TO UPTAKE OR ACCUMULATE NEONICOTINOID INSECTICDES

5.1 Introduction

Globally, wetlands and their associated macrophyte communities are documented to provide numerous ecosystem services such as regulation of water flows, life cycle maintenance, food, water purification, and CO_2 regulation (Engelhardt and Ritchie, 2001; Bornette and Puijalon, 2011; Clarkson *et al*., 2013). Macrophytes have the ability to modify both wetland physical structure and environmental conditions (van der Valk, 2012). Physically, the plants slow erosion, reduce sedimentation, filter contaminants and organic inputs, and provide a surface for microorganisms to grow (Vermaat *et al*., 2000; Rejmankova, 2011). Metabolically, aquatic plants release oxygen into wetlands and regulate fluxes of nutrients (Brix, 1994; Lin *et al*., 2002). Macrophytes are also biologically important as refuge and food for numerous wetland-dependent organisms such as amphibians, invertebrates, and water birds.

Standing wetland water is often "buffered" from anthropogenic impacts by the presence of zonal aquatic plant communities organized along water depth gradients (Stewart and Kantrud, 1972; Guntenspergen *et al*., 2002). Different wetland plants along this gradient have the capability to mitigate a range of contaminants from entering surface waters (Maillard *et al*., 2011). Previous studies of macrophytes have demonstrated their potential to uptake a range of contaminants including metals, explosives, and organic pollutants (Dhir *et al*., 2009; Gregoire *et al*., 2009). Specifically, plants can buffer water from pesticides through sorption or degradation in environments rich in organic matter and biological activity (Carluer *et al*., 2011). Buffers ranging in size from 10-30 m reduced a suite of aqueous and particulate concentrations of pesticides by >50% (Dunn *et al*., 2011). In agroecosystems such as the Prairie Pothole Region, the ability of wetland plants to mitigate or uptake chemicals such as pesticides may greatly reduce the amount of chemical inputs entering surface water systems. Aquatic plants may immobilize or extract pesticides through surface adsorption and/or uptake by the roots (Gregoire *et al*., 2009). Plants common to Prairie wetland ecosystems such as *Typha latifolia* are previously shown to uptake metalaxyl and simazine (Wilson *et al*., 2000) and efficiently removed parathion from water and sediments (Amaya-Chávez *et al*., 2006). However, pesticide sequestration by

aquatic plants depends on the species of interest, biochemical composition of plant tissues, and physico-chemical properties of the contaminant (Dhir *et al*., 2009).

Neonicotinoids are a systemic insecticide widely used as a seed treatment across agro-wetland landscapes such as the Prairie Pothole Region, Great Plains, and the Midwestern United States. Registered for use in 120 countries, active ingredients such as clothianidin, imidacloprid, and thiamethoxam are applied to protect major crops (e.g., oilseed rape, cereals, fruit and vegetables) to protect against sucking and chewing insect pests (Elbert *et al*., 2008). However, because of their high solubility in water, detectable levels of neonicotinoids have been found in numerous global surface water systems such as rivers and wetlands (Starner and Goh, 2012; Hladik *et al*., 2014; Main *et al*., 2014; Smalling *et al*., 2015). They are equally persistent in agricultural soils (DeCant and Barrett, 2010; Goulson, 2013; Bonmatin *et al*., 2015) and can accumulate in soils over time (Jones *et al*., 2014). Neonicotinoid concentrations reported in field margin vegetation range from 1 to 9 ppb (Krupke *et al*., 2012); however, studies of vegetation uptake are limited.

As neonicotinoid use continues to grow in both use and spatial extent (Main *et al*., 2014; Douglas and Tooker, 2015), it is important to identify potential strategies to reduce neonicotinoid contamination of surface water systems. Although buffer presence can slow or reduce contaminant infiltration into surface waters, buffers alone may not be adequate for reducing contaminant transport into wetlands (Skagen *et al*., 2008). In an exploratory analysis of ecological and landscape drivers of neonicotinoid detections and concentrations in Prairie wetlands, we found that specific wetland plant species such as *Mentha arvensis* (wild mint) were more likely associated with lower neonicotinoid water concentrations, while other species (e.g., *Scirpus validus*, softstem bulrush) were associated with higher neonicotinoid concentrations (Main *et al*., 2015). However, it was not clear whether this was attributed to the macrophytes ability to uptake the pesticide out of the water or whether the plant community are acting as a physical barrier to transport. The ability of wetland macrophytes to either uptake or buffer neonicotinoid insecticides in surface waters could offer an important mitigation strategy in agroecosytems. Therefore, our objective was to determine the degree of neonicotinoid uptake and accumulation by Prairie wetland macrophytes both under experimental conditions and in natural wetlands under varying exposures. We propose 2 non-mutually exclusive hypotheses

about macrophyte function in Prairie wetlands: 1) plants are able to uptake neonicotinoid active ingredients into their tissues; and 2) plants act as a barrier and/or mitigate against neonicotinoid movement into vegetated wetlands.

5.2 Methods

5.2.1 Macrophyte uptake of neonicotinoids in microcosms

We conducted an outdoor microcosm experiment beside the University of Saskatchewan's agricultural greenhouses in June of 2014 to compare the neonicotinoid accumulation potential of two common Prairie macrophytes: *Alisma triviale* (Northern water plantain) and *Typha latifolia* (Broadleaf cattail). We chose *A. triviale* and *T. latifolia* for this experiment as they are locally abundant throughout the Prairie Pothole Region and representative of species common to the shallow marsh zones of wetlands. Additionally, the differences in leaf structure may provide alternative routes of sorption as *A. triviale* can have broad leaves either floating or submerged whereas cattails have long spikes. Previous research has identified both species as being associated with high neonicotinoid detections, but often found in wetlands with differing levels of concentration (Main *et al*., 2015).

Preparation of neonicotinoid dosing solutions

Our dosing solution was made by leaching thiamethoxam-treated canola seeds (Helix Xtra® Syngenta) in 1 L of deionized water. Target concentrations were based on both an application rate and a potential rainfall event. We used a typical product application rate (24.2 g a.i./ha) and then calculated the potential concentration entering a wetland after a 5 mm rainfall event across one hectare planted to treated seed (a worst-case scenario of 100% run-off entering a wetland system). We further considered previously determined peak neonicotinoid concentrations detected in Saskatchewan wetlands (mean: 0.110 µg/L; max: 3.1 µg/L) to determine final dilutions (Main *et al*., 2014). Microcosms were exposed to three environmentally relevant concentrations (i.e., concentration of a pesticide likely to affect ecological characteristics of an exposed system) of the neonicotinoid insecticide, thiamethoxam (0 µg/L, 0.380 µg/L, 3.8 µg/L). First, We created a nominal stock solution target of 0.484 mg/L by leaching 0.12 g, 0.24 g, and 0.48 g of treated-seed in three separate, chemically-cleaned (acetone:hexane) amber glass jars (VWR International) in 1L of deionized water. Bottles were

shaken by hand for ~5 min, covered with aluminum foil, and placed in the lab cooler at 4°C for 24 hours. The following day, each jar of eluate was filtered of its seeds and transferred to a new chemically-cleaned amber jar and again stored at 4°C until analysis. Actual thiamethoxam concentrations were measured for each of the 3 stock solutions in triplicate. All samples were sent to the National Hydrology Research Centre, Environment Canada, Saskatoon, Saskatchewan (see section below on chemical analysis) within 48 hr of the subsample being collected. Our average measured concentrations of the three stock solutions were as follows: 0.12 g seed/L = 0.380 mg THX/L; 0.24 g seed/L = 0.827 mg THX/L; and, 0.48 seed/L = 1.84 mg THX/L. We used the 0.380 mg THX/L stock to prepare a high (100x dilution; 20 ml = 3.8 µg/L) and low-dose treatment (1000x dilution; 2 ml = 0.380 µg/L) for our microcosms.

Plant and sediment collection

Typha specimens, and sediment were collected on June 2, 2014 from the same seasonal (Stewart and Kantrud, 1971) agricultural wetland near Clavet, Saskatchewan (51° 58′ 44.4″ N, 106° 19′ 51.6″ W). Previous years' seeded crops were untreated (B. Blackmore, Goodale Farms personal communication), and included typical rotations of alfalfa and barley. Multiple water samples collected from the source wetland (2013, 2014) indicated no detectable levels of neonicotinoid insecticides. Neonicotinoids have previously been shown to persist in agricultural soils (Bonmatin *et al*., 2005a; Jones *et al*., 2014), but had little to no detections in wetland sediment (Main *et al*., 2014). Sample macrophytes (~ 70 cattails; mean height = 27 cm) were transported to the greenhouse site in 50 L Rubbermaid containers holding enough water to cover plant roots. Upon arrival, they were rinsed with tap water to remove excess sediment, periphyton, and any attached invertebrates.

Microcosm design and set-up

Wetland microcosms were constructed using 4 L pails where ~1 L of wet weight, field-collected sediment (~ 1 kg or 4.5 cm depth) was added to each pail as a substrate layer. A single cattail or water plantain was randomly taken from the Rubbermaid bins and placed in the centre of the sediment where the planting medium was positioned to completely cover their rhizome/roots. Initial plant height, number of shoots and a qualitative assessment of health (i.e., leaf color, discoloration) were recorded at the time of planting. We added 250 ml of de-

chlorinated tap water to each pail to slowly acclimate plants to the microcosm. An additional 250 ml of de-chlorinated tap water was added to each microcosm on day 2, 4, 6, and 8 until a final water depth (~ 2 L) of 8 cm was achieved. On day 10, all microcosms were equalized to a final combined sediment/water depth of ~12.5 cm or 3 L. In addition, standard water parameters of pH, conductivity, temperature, dissolved oxygen (YSI Professional Plus handheld meter) were collected from random microcosms on day 0, 24 hr, and 7 d.

Dosing of microcosms

Plants were given a ten day equilibrium period prior to insecticide application. We completed a dosing treatment at three different levels: control (water only), low dose (0.380 µg/L), and high dose (3.8 µg/L) and collected samples at two intervals (24 hr and 7 d) post treatment. Each of the dosing groups and controls were replicated 10 times for a total of 60 microcosms per plant species. Six additional microcosms of sediment and water only (control = 2, low dose = 2, high dose = 2) were also included in the experiment to test thiamethoxam degradation rates in systems without plants. Microcosms were each dosed once on day 0 beginning in the evening to reduce the potential for any photolytic breakdown. We began applying thiamethoxam, starting with insecticide-free controls at 21:00 h and finished with the 3,800 ng/L treatments at 22:00 h. Controls were treated with 2 or 20 ml of deionized water. *T. latifolia* microcosm experiments were run two weeks before beginning the *A. triviale* study. Although we attempted to maintain nominal dosing levels at 3.8 µg/L (high) and 0.380 µg/L (low), our dose groups differed for both experiments due in part to seed leachability and dose correction. The initial high dose in the *T. latifolia* microcosms was measured at 4.1µg/L and the initial high dose in *A. triviale* microcosms was 4.8 µg/L. There was some low level contamination of control buckets as indicated.

Measurements of microcosm water and plants

Measurements of water depth, plant height (cm), and number of leaves were recorded from all microcosms before and after plant extraction. We also measured temperature changes in the microcosms during the 7 d dosing period using two HOBO data loggers. No additional water was added after dosing began; however, the microcosms were outdoors and subject to natural changes in weather and rainfall. For the *Typha* microcosms, 4.2 cm of rain fell between the

dosing period on June 12 and collection date on June 19 (Day 7) with the average temperature in the microcosms being 14.8 °C (max: 30.2 °C). *Alisma* microcosms received 3.7 cm of rainfall before dosing and < 1cm after dosing began with an average microcosm temperature of 15.7 °C (max: 27.2 °C).

Termination of experiment

Plants were removed from their respective microcosms at intervals of 24 hr and 7 d after dosing. Upon termination, the rhizomes/roots of extracted plants were gently washed in de-chlorinated tap water to remove any build-up of sediment. The entire plant was placed in a large polyethylene bag and frozen at -20°C for future analysis. A 250 ml water sample of each dose group was randomly collected from three microcosms at the harvesting intervals for neonicotinoid analysis.

5.2.2 Vegetated and unvegetated wetland site selection

We examined the influence of vegetation presence on mitigation of neonicotinoid wetland contamination through testing of neonicotinoids in vegetated and unvegetated wetlands. Macrophyte uptake and accumulation were also observed under actual field conditions, by measuring concentrations of neonicotinoids in multiple plant species. In late spring of 2015, we selected 20 representative agricultural wetlands (10 vegetated; 10 unvegetated) situated in fields under clothianidin-treated canola production near Alvena, Saskatchewan (52.5167° N, 106.0167° W). Both vegetated and unvegetated were managed by the same landowner and field applications, crop (canola), and timing of seeding were constant between study wetlands. The wetlands chosen encompassed a range of wetland classes (seasonal, semi-permanent, permanent) and their likelihood of producing a zonal macrophyte community was based on wetland assessment data from 2012-2013 (Main *et al*., 2015). We previously defined a non-buffered (i.e., unvegetated) wetland in two ways: 1) the wetland was completely devoid of vegetation; and, 2) the wetland vegetation was highly disturbed and left numerous openings to the surface water. In this study area, both types were identified. Vegetated wetlands typically contained continuous, concentric rings of vegetation which exhibit the more classical patterns of plant zonation found in undisturbed Prairie wetlands (Guntenspergen *et al*., 2002). Because the extent to which agricultural wetlands may be disturbed in a given year is often based on their size, depth, and

formation post-snowmelt, we did not seek to have a minimum vegetation zone width as part of our selection criteria. Instead, we focused on visual continuity of vegetation surrounding the wetland of interest.

5.2.3 Collection of wetland water and wetland plants

Water samples were collected from both vegetated and unvegetated wetlands at four time periods during our eight week study: pre-seeding (May 1); post-seeding (May 28); and, twice during the canola growth period (June 12, June 26). At each wetland, we used two chemically-cleaned (acetone:hexane washed) 1 L amber glass jars to collect a separate subsurface grab sample at a depth of 10-15 cm near the wetland edge, and central portion of the pond. Bottles were sealed with Teflon-lined caps, stored in the dark during transport, and refrigerated at 4 °C until analysis. In addition, we further recorded wetland water depth measurements near the edge and in the central portion of the study ponds. We used a handheld digital water quality meter (YSI Professional Plus) to document standard water parameters (temperature, dissolved oxygen, conductivity, salinity, pH) at each site and calculated wetland area, and elevation using a Garmin (Montana 650) GPS.

To identify the ability of wetland plant species to potentially mitigate neonicotinoid movement into Prairie wetlands and/or accumulate neonicotinoid active ingredients into their tissues, we collected a diversity of plant samples from a subset of five vegetated wetlands (Fig. 5.1).

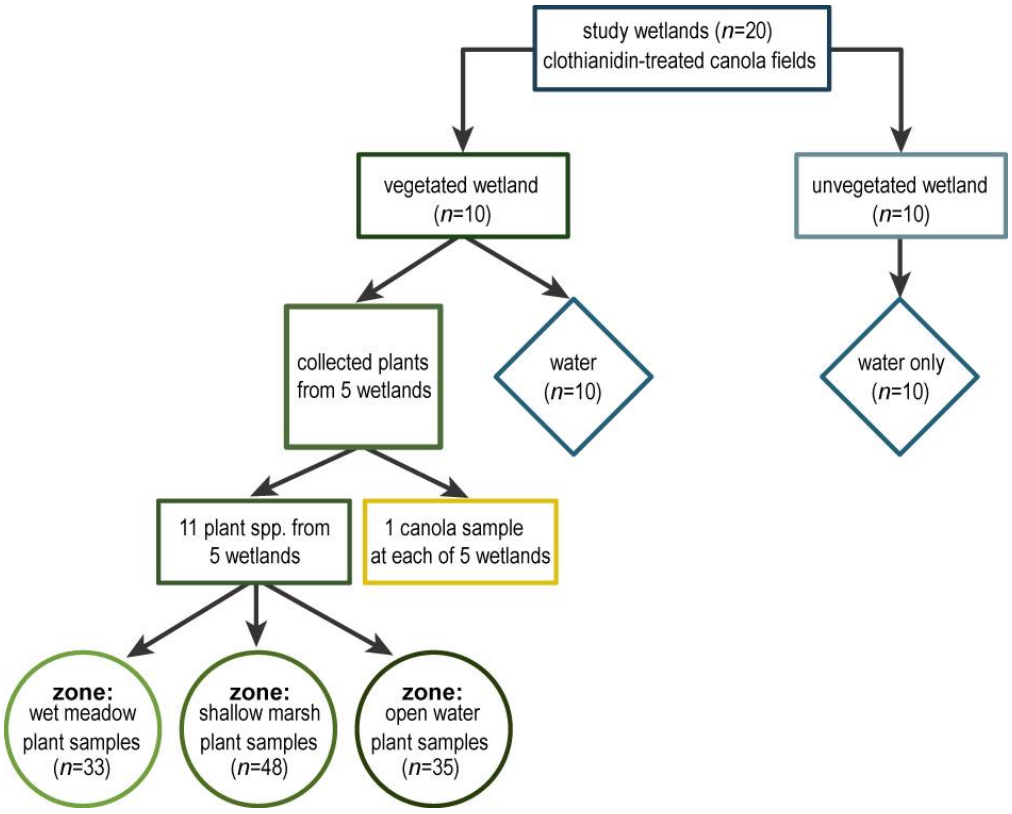

Figure 5.1 Flowchart of sampling strategy for collection of wetland water and macrophytes from vegetated wetlands and collection of water only from unvegetated wetlands situated in clothianidin-treated canola fields.

Where possible, we sought to collect a minimum of five different plant species of commonly identified Prairie macrophytes found in – or extending into – the wet meadow or shallow marsh zone. We used this edge to delineate the biological extent of the wetland (Kantrud and Newton, 1996), and to identify the plants most likely to initially mitigate or slow potential surficial run-off containing insecticide residues. To act as a positive control, surrounding treated canola plants were also sampled at each of the five vegetated wetlands. Macrophytes were collected from vegetated wetlands beginning four weeks after seeding and then every two weeks until the end of the eight week study. We consistently sampled *Alisma*, *Equisetum*, and *Typha* from almost all vegetated wetlands. To a lesser extent, we opportunistically collected samples of *Carex* and *Sparganium*, *Phalaris arundinacea*, *Mentha arvensis*, *Hordeum jubatum* and *Beckmannia syzigachne*. As 11 of 20 (vegetated and unvegetated) study wetlands had a developed submerged

aquatic plant community, the presence of floating aquatic plants, or had the formation of algal blooms, we opportunistically collected these samples from the shallow open water zone (Stewart and Kantrud, 1971). Our goal was to further identify mitigation potential of plants whose life cycle was either mostly or completely under water. All plant samples were taken from the same zone and general position of the wetland throughout the study. We removed the entire plant from the soil, gently washed the specimen, and then pooled approximately 5 plants of each species collected forming a composite sample. Composite samples were placed in polyethylene bags and stored in a cooler to be transported back to the lab. We recorded sample plant height, number of leaves, and wet weight for each composite sample. Plant shoots and roots were thoroughly rinsed with tap water before being stored frozen at -20 °C. The width of the wetland vegetation zones at four compass points surrounding the vegetated wetland was also calculated during the study period (Spencer *et al*., 1998).

5.2.4 Neonicotinoid chemical analysis: Dosing solutions, wetland water and plant tissues

Wetland water, microcosm water, and plant tissue samples were analyzed at the National Hydrology Research Centre, Environment Canada, Saskatoon, SK. Water samples were analyzed using methods previously published in Main *et al* (2014). All neonicotinoid concentrations were recovery corrected; all laboratory and field blanks were below detection.

Analytical standards of thiamethoxam (ISTDx2 = Thiamethoxam-d_3), clothianidin, and imidacloprid-d_4 were from Sigma-Aldrich, Chem Service (West Chester, PA, USA), and CDN Isotopes (Pointe-Claire, Quebec, CA), respectively. Plant tissue samples were analyzed using a multi-step QuECHERS method. Plant tissue samples were prepared in the lab by washing a second time, rinsing any residual sediment off the plant/roots using tap water followed by an additional rinse of Milli-Q water. Tissue samples were cut into ~2.5 cm pieces which were then placed in storage bags and re-frozen at -20 °C. We used a food processor to homogenize the frozen whole plant samples (~100 g), with dry ice added if the sample was too wet to grind. A subsample of this tissue was used to calculate a wet weight. QA/QC tissue samples for each plant species were previously homogenized. 10 g of tissue was added to a 50 ml centrifuge tube and spiked with analytes (60 ppb). Again, we measured ~ 10 g of sample into a 50 ml centrifuge tube and noted the weight for final calculations. We added 15 ml acetonitrile (ACN) to the tube which were then shaken intensively for ~1 min. Four grams of anhydrous $MgSO_4$ and 1 g of NaCl were

added for the liquid/liquid partition and again shaken intensively for ~ 1 min. Tubes were centrifuged at 4500 rpm for 7 min followed by 6 ml of aliquot supernatant being placed into a 15 ml cleanup tube (Thermo Scientific – 60105 – 205 – 900 mg $MgSO_4$ / 300 mg CUPSA / 150 mg CUCARB) and shaken intensively for ~ 1 min. Tubes were centrifuged at 5000 rpm for 2 min. We then placed a 3 ml aliquot of the supernatant into a test tube. A stream of nitrogen was used to facilitate drying of the sample. Samples were then reconstituted by adding 100 µL CAN and 400 µL Milli-Q water using a vortex to mix, sonicated for ~1 min and transferred to a total recovery vial. 10 µL of 2.5 ppm ISTDx2 were added to samples and mixed using a vortex mixer. Approximately 48 hr after wet weight was measured; samples were re-weighed to calculate a dry weight. Final extracts were analyzed directly through use of LS-MS/MS.

Due to the high diversity of plant species collected as part of our microcosm and field study, limits of detection and quantification varied by plant species and in water as listed in Table 5.1.

Table 5.1 Summary of limits of detection (LOD) and limits of quantification (LOQ) for plant species collected from vegetated wetlands, microcosms, and wetland and microcosm water. Concentrations in plant tissues are in micrograms per kilogram (µg/kg) and micrograms per liter (µg/L) in water.

	Imidacloprid (µg/kg)		Clothianidin (µg/kg)		Thiamethoxam (µg/kg)	
Natural wetland plant species	**LOD**	**LOQ**	**LOD**	**LOQ**	**LOD**	**LOQ**
T. latifolia	0.2	0.6	0.2	0.5	0.5	1.5
A. triviale	0.4	1.2	0.5	1.5	0.4	1.3
E. arvense	0.29	0.87	0.22	0.67	0.45	1.35
H. jubatum	0.46	1.37	0.58	1.73	0.13	0.4
B. syzigachne	0.23	0.69	0.25	0.76	0.54	1.61
S. eurycarpum	0.2	0.59	0.26	0.79	0.38	1.14
P. pusillus	0.25	0.75	0.14	0.42	0.39	1.18
Algae spp.	0.27	0.8	0.17	0.5	0.4	1.2
P. richardsonii	0.19	0.56	0.2	0.61	0.47	1.42
P. arundinacea	0.2	0.6	0.2	0.7	0.6	1.7
M. arvensis	0.55	1.65	0.66	1.99	0.1	0.31
Canola	0.47	1.41	0.35	1.04	0.55	1.64
Microcosm plant species						
T. latifolia	1.4	4.3	1.5	4.6	2.1	6.3
A. triviale	1.6	4.9	2.6	7.8	2.8	8.3
Microcosm/Wetland water (µg/L)	0.001	0.004	0.002	0.005	0.002	0.007

5.2.5 Statistical analysis: Effect of vegetation presence on wetland concentrations

We analyzed the effects of vegetation presence and sampling period on change of total neonicotinoid concentration in wetlands over time using a Gaussian general linear mixed model (GLMM) in package "nlme" (Pinheiro *et al*., 2014) in R 3.1.1 (R Core Team, 2014). We split the wetlands into vegetated and unvegetated categories based on the presence or absence of wetland zonation during field data collection. We predicted that as the presence of diverse wetland species increased throughout the summer, wetland concentrations would decrease over time and/or be lower than those detected in unvegetated wetlands. Vegetation presence and time (sampling period) were fixed effects and wetland ID was included as a random effect to account for repeated measures. Model fit was assessed through a combination of visual inspection of

residuals and a Shapiro-Wilk test to assess the fit of residuals to a normal distribution (Michel, 2014). We further assessed temporal autocorrelation in our repeated measures model by calculating partial autocorrelation functions, and we allowed variances to differ between dates to account for heterogeneity in model residuals (Michel, 2014). We used an exhaustive model selection framework based on Akaike's information criteria (AIC; Akaike, 1987) to determine inclusion of fixed and random effects.

5.3 Results

5.3.1 Thiamethoxam uptake by *A. triviale* and *T. latifolia*

Thiamethoxam concentrations in the *T. latifolia* microcosms differed slightly with a ~30% loss of the active ingredient after 24 hrs in water sampled from both high and low dose groups. Thiamethoxam loss increased to ~70% in both dose groups by day 7. Similar rate loss ~70% of the active ingredient occurred in the *A. triviale* microcosms after 24 hrs in both dose groups and upwards to 92% by day 7 in the high dose group. In water and sediment only microcosms, an initial concentration of 4.11 µg/L experienced an almost 95% loss of active ingredient by day 7, with similar results in the water and sediment controls during the *Alisma* experiment (Table 5.2). We did not extract sediment samples from microcosms during the experiment.

Table 5.2 Summary of thiamethoxam concentration (µg/L) by treatment (control, low, high)* measured in microcosm water and sediment only treatment.

	Typha latifolia			*Alisma triviale*		
	Water Conc. (sediment + water treatment only)			Water Conc. (sediment + water treatment only)		
	Day 0	24 hr	Day 7	Day 0	24 hr	Day 7
Control	0	0.007	0	0.006	0.008	0
Low	0.335	0.253	0.048	0.568	0.231	0.089
High	4.11	2.97	0.171	4.81	2.65	0.339

*Nominal concentrations were as follows: high dose (3.8 µg/L) and low-dose (0.380 µg/L).

Plants remained healthy throughout the experiment and exhibited growth over the 7 days. Mean *Typha* height increased from 40.7 cm on day 0 to a final mean height of 46.5 cm on day 7

(Table 5.3). Mean *Alisma* height increased from 25.5 to 33.7 cm by the end of the experiment while developing inflorescence. Due to precipitation (i.e., daily rainfall) during the *Typha* experiment, microcosms experienced an average gain of 3.3 cm of water from day 0 to day 7. *Alisma* microcosms experienced steady temperature increases with most microcosms subsequently losing water (initial depth, 8cm; mean day 7 depth = 2.8 cm; Table 5.3).

Table 5.3 Summary of water quality and growth parameters measured during the microcosm dosing experiment for both *T. latifolia* and *A. triviale*. Each measurement is shown as a mean value for that sampling period.

	***Typha* Microcosms**							
	pH	Temp (°C)	DO (mg/L)	Cond. (µS/cm)	Salinity (ppt)	Depth (cm)	Height (cm)	No. of shoots or leaves
Day 0	8.1	18.1	6.9	527.3	0.26	8.0	40.7	5.6
24 hr	7.4	14.3	4.6	515.6	0.26	7.3	43.2	6.1
Day 7	7.7	18.8	5.7	368.4	0.18	11.3	46.5	7.5
	***Alisma* Microcosms**							
	pH	Temp (°C)	DO (mg/L)	Cond. (µS/cm)	Salinity (ppt)	Depth (cm)	Height (cm)	No. of shoots or leaves
Day 0	7.7	17.8	6.2	284.8	0.13	8.0	25.0	7.3
24 hr	7.2	19.7	n/a	297.4	0.14	7.7	26.7	8.6
Day 7	7.8	21.7	5.8	405.2	0.22	2.8	33.7	7.7

n/a = a stable dissolved oxygen reading was unavailable after multiple attempts

In both *T. latifolia* and *A. triviale microcosms*, most plant samples showed positive detections (i.e., >LOD <LOQ) of thiamethoxam in their respective tissues (Table 5.4). Neither plant species contained quantifiable thiamethoxam concentrations (>LOQ) during our experiment.

Table 5.4 Summary of arithmetic means ±SD of thiamethoxam concentration (µg/L) by treatment (control, low, high)* measured in microcosm water and detections (%) in plant tissues from uptake experiments with *Typha* and *Alisma*.

	Typha latifolia					*Alisma triviale*				
	Water Conc. µg/L (mean ± SD)			Plants (% detections)*		Water Conc. µg/L (mean ± SD)			Plants (% detections)*	
	Day 0	24 hr	Day 7	24 hr	Day 7	Day 0	24 hr	Day 7	24 hr	Day 7
Control	0	0.016 ± 0.004	0.019 ± 0.017	ND	ND	0.006	0.025 ± 0.025	0.019 ± 0.014	ND	ND
Low	0.335	0.238 ± 0.006	0.109 ± 0.011	ND	ND	0.568	0.203 ± 0.041	0.089 ± 0.036	ND	ND
High	4.11	2.84 ± 0.494	0.993 ± 0.149	4 (100%)	5 (100%)	4.81	1.53 ± 0.193	0.384 ± 0.067	5 (63%)	8 (100%)

*Nominal concentrations were as follows: high dose (3.8 µg/L) and low-dose (0.380 µg/L).

5.3.2 Effect of vegetation presence on natural wetland concentrations

We detected neonicotinoids more often in water collected from unvegetated wetlands (87.5%) than vegetated ponds (75%) in our study. Mean water concentrations in both unvegetated and vegetated wetlands remained relatively low during this study and were not statistically different by type (β ± S.E.: -0.49 ± 0.37, $P = 0.19$) although peak concentrations were higher in unvegetated wetlands (Table 5.5). Wetland type and time explained 13% of the variation ($R^2 = 0.13$) in our model of neonicotinoid concentrations from pre-seeding to mid-growing season. Our interaction model (wetland type x time) revealed a significant interaction between presence of vegetation and time such that total neonicotinoid concentrations decreased over time in vegetated wetlands ($X^2 = 16.36$, $P = <0.0001$) but not in unvegetated wetlands ($X^2 = 2.09$, $P = 0.148$; Fig. 5.2).

Table 5.5 Summary of percent detections, arithmetic means, and maximum concentrations of total summed neonicotinoids (clothianidin, imidacloprid, thiamethoxam) in water from wetlands situated in canola fields. Concentration values are measured in µg/L and presented as overall summaries by week for each wetland type: unvegetated or vegetated.

Wetland Type	Week	Detection (%)	Neonicotinoid Concentration: Mean ± S.E.	Max. Concentration
unvegetated	1	90	0.018 ± 0.005	0.047
n = 10	4	90	0.010 ± 0.002	0.019
	6	90	0.127 ± 0.059	0.459
	8	80	0.008 ± 0.002	0.019
vegetated	1	100	0.014 ± 0.002	0.022
n = 10	4	50	0.008 ± 0.003	0.019
	6	80	0.012 ± 0.002	0.026
	8	70	0.005 ± 0.001	0.013

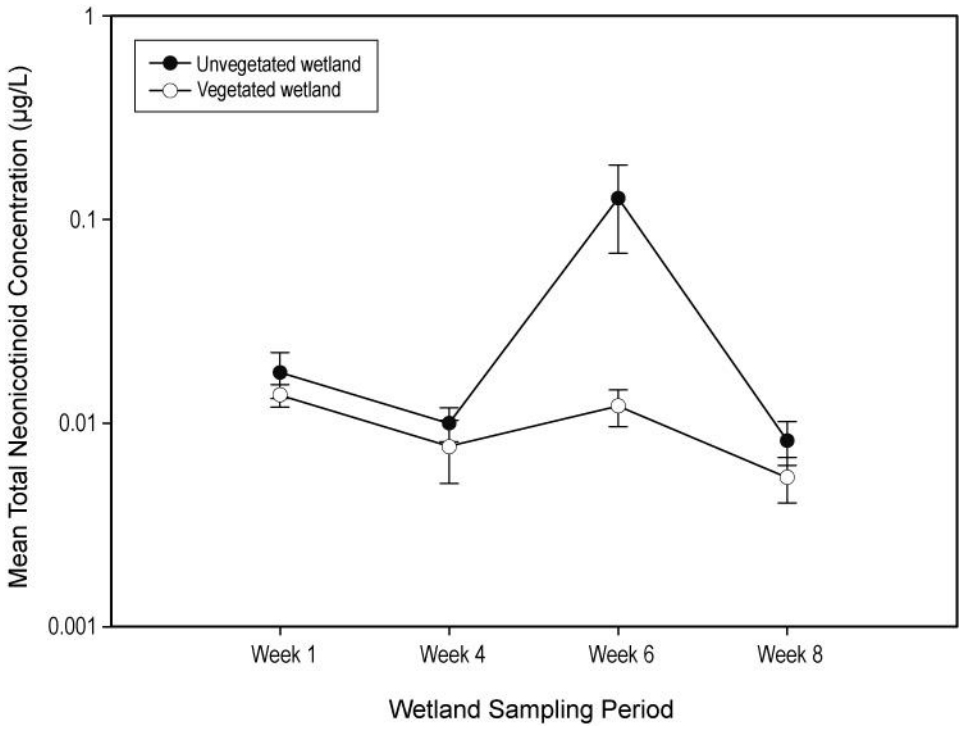

Figure 5.2 Change in mean (±SE) total neonicotinoid concentration (µg/L) in wetland water over time by wetland type: unvegetated (n = 10) or vegetated (n = 10). Mean concentrations are presented on a log scale. Seeding completed after Week 1, but before Week 4.

5.3.3 Uptake of neonicotinoids by plant species found in vegetated wetlands

To evaluate the number of neonicotinoid detections in plant tissues, we combined all trace detections regardless of active ingredient. Of the 11 wetland plant species collected from the five vegetated wetlands, six species contained positive trace neonicotinoid detections defined as >LOD and ≤ LOQ (Table 5.1). The highest number of trace positive detections was found in *Equisetum arvense* (93%) with the lowest number in *Sparganium eurycarpum* (20%; Table 5.6).

Table 5.6 Summary of trace positive detections and concentration ranges (µg/kg) of individual neonicotinoids in clothianidin treated canola and wetland plant tissues collected from vegetated wetlands and surrounding agricultural fields in Saskatchewan. Trace (Tr) indicates a positive detection (>LOD <LOQ) <LOD = below limit of detection. LOD and LOQ are the same as Table 5.1

Plant species	*n* wetlands	*n* plant samples (composite)	No. samples >LOD <LOQ	No. samples >LOQ	% positive detections	Imidacloprid (Range: µg/kg)	Clothianidin (Range: µg/kg)	Thiamethoxam (Range: µg/kg)
Typha latifolia	5	15	8	1	**60**	**<LOD - 2.61**	<LOD	**<LOD - 8.44**
Alisma triviale	4	11	6	1	**64**	**<LOD - 2.51**	<LOD	<LOD
Equisetum arvense	5	14	9	4	**93**	<LOD	**<LOD - 2.01**	<LOD
Hordeum jubatum	1	1	0	0	**0**	<LOD	<LOD	<LOD
Beckmannia syzigachne	1	1	0	0	**0**	<LOD	<LOD	<LOD
Sparganium eurycarpum	2	5	1	0	**20**	<LOD	<LOD - Tr	<LOD - Tr
Phalaris arundinacea	1	1	0	0	**0**	<LOD	<LOD	<LOD
Mentha arvensis	1	1	0	0	**0**	<LOD	<LOD	<LOD
Potamogeton pusillus	3	8	2	0	**25**	<LOD - Tr	<LOD	<LOD
Potamogeton richardsonii	1	2	0	0	**0**	<LOD	<LOD	<LOD
Algae spp.	2	4	2	0	**50**	<LOD - Tr	<LOD	<LOD
CLO-treated Canola	5	5	0	5	**100**	<LOD	**2.37 - 22.9**	**<LOD - 12.34**

Only *Typha*, *Equisetum*, and *Alisma* had tissue concentrations that were >LOQ for wetland plants with *E. arvense* concentrations above the limit of quantification in more than one composite plant sample. In all wetland plants collected in our field study, *T. latifolia* contained the highest quantifiable concentration of 8.44 µg/kg. Clothianidin-treated canola plants collected from the agricultural field surrounding our study wetlands all held quantifiable neonicotinoid concentrations in plant tissues up to 22.9 µg/kg (Fig 5.3).

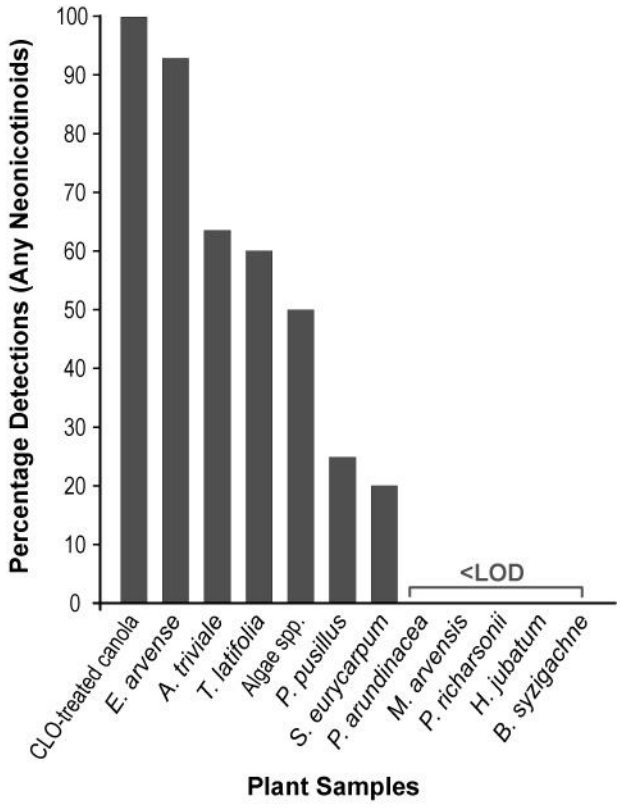

Figure 5.3 Percentage positive detections (>LOD) of any neonicotinoid active ingredient (clothianidin, imidacloprid, thiamethoxam) in canola and wetland plant tissues from samples collected from vegetated wetlands situated in canola fields of Saskatchewan. <LOD = below limit of detection.

5.4 Discussion

Wetland plant species have been identified as potential indicators and/or drivers of both neonicotinoid detections and concentrations in Canadian Prairie wetlands with highly disturbed wetlands equating to higher probability of neonicotinoid detection (Main *et al*., 2015). Plants can affect wetland water concentrations by accumulating chemical transport, slowing pesticide movement, and influencing retention time needed for chemical degradation (Stehle et. al., 2011). In this study, we found that wetlands surrounded by vegetation were more likely to have neonicotinoid concentrations that decreased slightly over time suggesting a small mitigation effect of transport. Additionally, some common Prairie wetland species demonstrate the ability to uptake neonicotinoid active ingredients under natural conditions. Numerous global surface water systems have detectable levels of neonicotinoids (Morrissey *et al*., 2015), but few studies, have attempted to quantify the potential for adjacent aquatic vegetation to mitigate neonicotinoid

movement into water bodies or uptake active ingredients. There is also a paucity of published studies comparing experimental data of neonicotinoid uptake against a field survey examining mitigation or uptake of neonicotinoid residues in wetland plant species. In our study, although plant microcosms were dosed with environmentally relevant concentrations of thiamethoxam (Main *et al*., 2014), only positive detections (> limit of detection < limit of quantification) were found in their respective tissues. In contrast, plants collected from wetlands situated in Saskatchewan agricultural fields were more likely to have a quantifiable level of a neonicotinoid active ingredient in their tissues with some readily accumulating the insecticide throughout the growing season.

5.4.1 Uptake of thiamethoxam by *Typha* and *Alisma* in an experimental setting

We found evidence of trace positive detections of thiamethoxam in both of our microcosm study species, but we could not quantify the extent to which our study species accumulated the active ingredient because of compound degradation and losses in water, and analytical limitations for detecting levels in plant tissues. Microcosms containing *Alisma* (~70%) experienced a higher loss of thiamethoxam after 24 hrs than microcosms containing *Typha* (~30%). Studies of neonicotinoid uptake by plants in experimental situations are limited; however, neonicotinoid insecticide residues have been detected in experiments using both wetland and terrestrial species. In a study of imidacloprid uptake by wetland plants of Suriname, 100% removal was observed for *Eleocharis mutata* and 86% removal by *Nymphaea amazonum* in mesocosms exposed to low target concentrations (60 µg/L) after 9 days (Mahabali and Spanoghe, 2014). Highest dose (1,000 µg/L) removal efficiency was on average 72% for the same period with up to 78.9% of the applied imidacloprid retained in plant tissues and roots of *N. amazonum* (Mahabali and Spanoghe, 2014). However, it is difficult to compare the results of our dosing study to the Suriname study. For example, the Suriname high dose experimental concentrations were over 250 times higher than our own study, the researchers dosed with imidacloprid which has a higher log Kow, the length of study was twice as long, and study plants used included different species such as water lilies and spikerushes. Additionally, the limits of detection for thiamethoxam in our microcosm plant tissues were 2.1 (*Typha*) and 2.8 µg/kg (*Alisma*) whereas for the Suriname study of chemically similar imidacloprid, detection limits were substantially lower at 0.01 µg/kg. Our ability to accurately detect quantifiable levels of

thiamethoxam in both study species may have been constrained by our analytical limits. Although terrestrial, *Plantago major* L. has similarly reduced imidacloprid concentrations in water by 55.8 to 95.1% during a 10 day exposure period with maximum concentrations in leaves (37.21 µg/gm) reduced to 1.46 µg/gm (Romeh, 2009). Thiamethoxam concentrations in summer squash grown with active ingredient application via drip irrigation were as high as 362 ppb for the whole plant (Stoner and Eitzer, 2012). In comparative experimental studies, the active ingredient dose far surpassed that of our own study at concentrations that were less likely to be environmentally realistic for the Canadian Prairies.

5.4.2 Do plants in Prairie agricultural wetlands mitigate and/or uptake neonicotinoids?

In this study, peak water concentrations were detected in wetlands with highly disturbed vegetation zones or those that were lacking intact vegetation communities. Our model comparing the influence of vegetation presence on wetland water concentrations over time indicated that vegetated wetlands are more likely to experience a greater decrease in neonicotinoid concentrations from seeding to the middle of the growing season. However, these results must be interpreted with caution as the interaction between vegetation presence and time only explained 13% of our overall model variance. We speculate that although wetland plants may provide a mechanism for neonicotinoid mitigation by slowing and/or removing these insecticides from being transported into Prairie wetlands, the surrounding vegetation may instead be altering the abiotic environment (van der Valk, 2012) or acting as a proxy for more complex interactions. Abiotic variables such as wetland depth, DOC, pH, and soil-water interactions may need further examination as they have the ability to influence pesticide concentrations (Wheeler, 2002). For example, DOC in Prairie wetlands can be highly variable (Waiser, 2006), but has also been shown to influence photolysis by acting as a photosensitizer (Zeng and Arnold, 2012). Higher concentrations detected in more disturbed wetlands may further be indicative of agricultural practices that seed through wetlands in drier years (Guntenspergen *et al*., 2002) which could act as a source of contamination during wet years from solubilisation of active ingredients.

Although not directly comparable, other wetland studies have shown that the presence of vegetation communities surrounding wetland water can mitigate a range of pesticides compared to unvegetated ponds (Bennett, 2006; Riens *et al.*, 2013). Most research has focused on the size of the vegetation community or "buffer" where a measured vegetation width is important to

reduce aqueous concentrations of pesticides (Dunn *et al.*, 2011). In contrast, we found that buffer width was unimportant in driving concentration changes in Prairie wetlands when compared to the presence of certain plant species (Main *et al.*, 2015). Individual plant species may be indicative of not only the ability to slow neonicotinoid transport, but are further able to accumulate varying concentrations of active ingredients into their tissues depending on the plant community present and structure of the plant itself.

In vegetated wetlands, three species (*Typha, Equisetum, and Alisma*) exhibited the ability to uptake quantifiable levels of the neonicotinoid active ingredients of imidacloprid, clothianidin, and thiamethoxam in wetlands situated in agricultural fields (Table 5.6). Although *Typha* did not effectively remove thiamethoxam from our microcosm experiment, a composite cattail sample (8.44 µg/kg, thiamethoxam) held the highest quantifiable detection of our field survey indicating the ability of this species to potentially phytoremediate neonicotinoids. In spite of the lack of wetland plant field data for comparison, neonicotinoids have been shown to accumulate in plants found in terrestrial areas. Clothianidin concentrations in field margin plants of up to 4 ppb in South Dakota milkweed plants (Pecenka and Lundgren, 2015) and 2.9 ppb (clothianidin) and 9.4 ppb (thiamethoxam) in dandelions (Krupke *et al.*, 2012) have also been detected. In a 2015 study of wildflowers surrounding seed-treated canola fields, 11.6% (imidacloprid) to 58.1% (thiamethoxam) of plant pollen samples contained a neonicotinoid active ingredient with concentrations up to 86 µg/kg (Botías *et al.*, 2015). It should be noted that both the limit of detection (0.12-0.16 µg/kg) and limit of quantification (0.36-0.48 µg/kg) used in Botías *et al.* (2015) are lower than our own for most species which may indicate our inability to determine appreciable uptake in wetland plants. Even though our plant tissue concentration values fall within the range listed above, the use of terrestrial study species precludes a direct comparison of results. Additional sampling of common wetland species may reveal other species with a greater ability to uptake these insecticides during annual agricultural production.

5.4.3 Potential of wetland plants for neonicotinoid contaminantion mitigation

Efficient removal of pesticides is further improved or inhibited by the plant species, the biochemical composition of the plant tissues and physico-chemical properties (e.g., solubility, pH, log K_{OW}, and K_{OC} coefficients; Dhir *et al.*, 2009). Higher likelihood of removal by plants is often restricted to pesticides with very low water solubility and very high log K_{OW} values

(Vymazal and Březinová, 2015). Indeed, pesticide adsorption by macrophyte roots may be avoided when log K_{OW} values are less than 0.5 (Stottmeister *et al*., 2003) and values less than 1 may further impede transport through plant membranes (Briggs *et al*., 1982). Thiamethoxam (-0.13), imidacloprid (0.57), and clothianidin (0.91) all have low log K_{OW} values making plant uptake more dependent on processes such as water transpiration rates, but high solubility may further leach compounds below the root zone (Tsao, 2003). As we did not measure the amount of water transpired by any studies species, it is difficult to identify how that may have influenced potential uptake of the active ingredient in our microcosm dose groups or field wetlands. Microcosm sediments were not sampled based on the field study results of Main *et al*. (2014) which indicated little to no partitioning of neonicotinoids to Prairie wetland sediments.

Aqueous photolysis of thiamethoxam (DT_{50}) can occur moderately fast at 2.7 d (Bonmatin *et al*., 2015) with a recent (and geographically similar) study in Winnipeg suggesting outdoor summer rates closer to 0.98 d (Lu *et al*., 2015). In both experimental plant groups our microcosms experienced a 70-90% loss of thiamethoxam over 7 d in water which may have occurred via increased photolytic breakdown due to high exposure to sunlight. However, in a study of thiamethoxam fate in microcosms planted with the submerged aquatic species, *Myriophyllum spicatum* (Eurasian watermilfoil), thiamethoxam degraded faster in systems with plants under both laboratory and outdoor conditions by indirectly increasing the rate of photolysis and hydrolysis (Traisup, 2012). By comparison, we collected samples of two submerged aquatic species (*Potamogeton pusillus* and *P. richardsonii*) from our vegetated wetlands. The pH levels of wetlands containing submerged species consistently increased over the sampling period (7.5 to 9.89) while concentrations decreased (or were absent by the end of study) suggesting agreement with the results of Traisup (2012). However, only trace positive detections were found in *P. pusillus* with no detection of an active ingredient in *P. richardsonii* plant tissues. Similar results have been demonstrated for the insecticide malathion where densities of *Elodea canadensis* (Canadian waterweed) reduced toxicity levels nine-fold as well as increased photosynthesis leading to a more alkaline pH for more rapid hydrolysis (Brogan and Relyea, 2014).

There is some disagreement about whether emergent macrophytes can influence pH changes (Wetzel, 2001), some species, such as the sedge *Eriophorum angustifolium* have been

shown to modify pH via root systems (Javed, 2011). In the present study, thiamethoxam levels in microcosms containing *A. triviale* were reduced by ~70% after 24 hours. Both the *Typha* and *Alisma* microcosms were situated in water with a mean pH range between 7.2 and 8.1, in which thiamethoxam is shown to be stable to hydrolysis (Guzsvány *et al*., 2006), but can increase more rapidly with increasing pH and temperature (Liqing *et al*., 2006). As some of our microcosms reached a pH of > 9 and a water temperature exceeding 30 °C, it is possible that both hydrolysis and photolysis led to increased rates of chemical breakdown in the water. It remains unclear if the presence of emergent macrophytes in field wetlands altered the abiotic environment enough to influence neonicotinoid degradation beyond some uptake of the insecticide itself.

The ability or inability to uptake neonicotinoids by the common wetland plants chosen for this study may further be species specific. Both of the species chosen for our microcosms as well as those selected in the field were based on likelihood to be situated in wetlands of high neonicotinoid detection probability and moderate to high concentrations (Main *et al*., 2015). We were further limited in the field survey based on consistent presence of species throughout the collection period as some plants (e.g., *Hordeum jubatum, Phalaris arundinacea*) were absent until later in the season. Little data is available on the phytoremediation capabilities of many of our species in relation to insecticides other than *Typha* and *Sparganium* spp. which were shown to effectively reduce experimental inflows of diazinon and permethrin (Moore *et al*., 2013). *Alisma* and *Equisetum* are further shown to tolerate polluted soils where they rapidly re-colonise contaminated sites (Desjardins *et al*., 2014) with *Equisetum* tolerant to heavy metals (Yoon *et al*., 2006). Of all species collected, *E. arvense* was the only plant found to have more than one quantifiable detection in its tissues (max: 2.01 µg/kg) and although farmers consider it a noxious weed, it may present a potential source of neonicotinoid uptake and mitigation. *T. latifolia* has been evaluated for hyperaccumulation of metals and nutrient reduction, but additionally for their potential to remediate herbicides such as simazine and atrazine (McKinlay and Kasperek, 1999; Wilson *et al*., 2000; Dhote and Dixit, 2009). A study of 2,4-D effects on *A. triviale* found that plants in five-leaf and scape elongation stages retained more of the compound than plants in the early flowering stage (Ransom *et al*., 1983).

The ability of a pesticide to sorb to plant tissues can be specific to certain types of plants and their ability to develop greater surface area below water (Elsaesser *et al*., 2011). In studies of

artificial wetlands, uptake of contaminants by numerous plant species may also be reflective of initial concentrations and hydraulic retention time (Gregoire *et al*., 2009). Our microcosm study concentrations, although environmentally relevant, may simply have been too low or for too short a duration for any quantifiable absorption by the species selected. In addition, our ability to detect quantifiable residues may have been further constrained by our analytical limits. However, the number of positive detections and higher quantifiable concentrations in field plant tissues may indicate the potential for reducing annual inflow of neonicotinoids into surface water systems such as wetlands. Additionally, because of the limited variance explained in concentration by vegetation presence, the likelihood that wetland plants are acting as a proxy measure for more complex biotic or abiotic interactions needs further explanation. Ultimately, further study is required to fully evaluate the effectiveness of wetland plants to reduce transport of neonicotinoids from surrounding agricultural fields and/or uptake neonicotinoids into their tissues.

5.5 Future Considerations

It is possible that our microcosm experimental design influenced the lack of quantifiable thiamethoxam detection in our wetland study species. Macrophytes may need a longer time period to uptake and partition neonicotinoids in their tissues and/or wetland plants may only uptake neonicotinoids during earlier stages of development. Unfortunately, experimental data are limited for uptake of neonicotinoids by wetland species (Mahabali and Spanoghe, 2014). However, some studies of plant uptake of pesticides outline a longer period of pre-experiment equilibrium (from weeks to months), dosing, and sampling (Runes *et al*., 2001; Bouldin *et al*., 2005; Dalton and Boutin, 2010). Our 10 day equilibrium period and 7 day dosing period may simply have been too short for this type of experiment to be effective. The artificial environment may further have been influenced by rainfall events, higher levels of sunlight and light reflection, and increased temperatures being situated outdoors near agricultural greenhouses which may have artificially heightened hydrolysis and photolytic breakdown. Other investigations of macrophyte uptake and mitigation of pesticides have employed a roof or shade-cloth to reduce environmental exposure to the elements (Brogan and Relyea, 2013; Mahabali and Spanoghe, 2014). Some plants exhibited stress through discoloration of leaves likely due to high temperatures and reflection of light. This could have inhibited their ability to uptake

thiamethoxam instead focusing on regulation of oxygen levels, photosynthesis, and thermal regulation. Neonicotinoids have been shown to alter plant growth and stress responses (Ford *et al*., 2011), but this is less likely the case in our experiment.

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CHAPTER 6: SYNTHESIS AND CONCLUSIONS

Understanding the numerous environmental factors that may be influencing the distribution, transport, and fate of neonicotinoid insecticides is becoming increasingly important as the impacts caused by chemical degradation of freshwater resources have become a global concern (Stehle and Schulz, 2015). Because of the controversy surrounding neonicotinoid use and their associated effects on non-target species, most research to date has focused on its potential role in pollinator declines and more recently on the toxicity to aquatic organisms (Blacquiere *et al*., 2012; Morrissey *et al*., 2015). However, the distribution, fate, and transport of these insecticides, particularly into sensitive aquatic ecosystems, have received less attention by comparison. Therefore, the purpose of this research was to explore ecological and landscape features of wetlands and how they influence neonicotinoid insecticide distribution and fate in Canadian Prairie wetland ecosystems. This research contributed to our understanding of current spatial distribution of neonicotinoid use across the Prairies, concentration ranges and frequency of detections of neonicotinoids in water and sediment in a subset of wetlands through time; identifying the major source of neonicotinoids to wetlands in spring; understanding the wetland and landscape features that most accurately predict neonicotinoid contamination; and, testing the ability of common wetland macrophyte to uptake and/or mitigate neonicotinoid residues entering surface waters.

In this thesis, I have provided the first evidence of the patterns of use in the agriculturally dominant areas of the Canadian Prairies and the major ecological and landscape drivers influencing neonicotinoid presence and concentrations in wetlands. New observational data further identified the key sources of neonicotinoid transport to wetlands in spring and the relative potential of aquatic macrophytes to take up neonicotinoids from the surrounding environment. Overall, the results of my dissertation have addressed several important gaps in the literature, but new interesting questions have also arisen about the specific ability of wetland plants to take up neonicotinoids and improve wetland water quality. Here, I summarize the findings of each chapter and their associated major conclusions followed by some recommendations for future research directions.

6.1 Synthesis, major findings, and limitations

6.1.1 Widespread use and frequent detection of neonicotinoid insecticides

Because of the lack of available pesticide sales and use data in Canada, it is difficult to accurately identify geographic areas of neonicotinoid use and thus the risk to adjacent aquatic systems such as wetlands. In Chapter 2, my study addressed the currently recognized need to spatially identify the geographic distribution of neonicotinoid use across the Prairies as well as provide new data on concentrations found in regional waterbodies (Goulson, 2013; Anderson *et al*., 2015). I demonstrated that almost half of the total Canadian Prairie cropland under production (2012) was seeded with a neonicotinoid-coated seed; with rapid growth in use of these seed treatments occurring in Canada with similar expansion in other regions such as the USA (Douglas and Tooker, 2015) and UK (Goulson, 2013). It should be noted that our estimates were conservative as specific geographic information of highest actual regional neonicotinoid use is not available, and remains unpublished. This may have influenced my ability to detect differences in loadings across a range of application rates being applied in this province (Saskatchewan). In some instances, even landowners (pers. comm.) were unwilling to discuss the amount of pesticide they applied to their lands and how often this occurred. However, field validation of wetlands situated in estimated high use and high wetland density areas contained a range of concentrations in wetland water samples, but with apparent infrequent detection in associated sediment. The number of detections and concentrations found in wetland water reflected sampling period (season), with highest detections occurring in spring but highest concentrations in summer. Perhaps most challenging (and intriguing) is that no single crop type was identified to most influence neonicotinoid concentrations in adjacent wetlands, suggesting the ubiquitous use of these insecticides on a wide range of crops.

6.1.2 Snowmelt transport of neonicotinoid insecticides to Prairie wetlands

Although peak neonicotinoid concentrations are typically found during the growing season (Hladik *et al.*, 2014; Main *et al.*, 2014; Schaafsma *et al.*, 2015), up to 91% of my initial study wetlands (Chapter 2) contained neonicotinoids in spring before seeding had occurred. This is a unique finding in pesticide environmental monitoring as water sampling efforts were completed shortly after initial ice-off where – in some cases – snow drifts could still be seen on

fields. In spring, I determined that meltwater, rather than snow or particulate, was most associated with initial neonicotinoid concentrations in wetland water. This study furthered our understanding of how neonicotinoids are mobilized into wetlands outside of summer months during high rainfall. During snowmelt, soluble pesticides can mobilize more quickly with a range of concentrations possible (Nicholaichuk and Grover, 1983; Waite *et al*., 1992; Meyer *et al*., 2011). Of the wetland water samples repeatedly collected from study fields - both previously treated and untreated - the majority contained at least one or more neonicotinoid active ingredients before agricultural production had begun regardless of field treatment. Temporary wetlands are critical resources for migratory birds and other organisms (Swanson *et al*., 1985; Fairbairn and Dinsmore, 2001), but were far more likely to have both higher neonicotinoid concentrations and increases in those concentrations over time which creates additional conservation challenges. Some pesticides (e.g., chlorpyrifos, atrazine) have been detected in marine ice (Chernyak *et al*., 1996), but it is unclear if neonicotinoids would behave similarly in permanent wetlands that freeze over winter as ice cores were unavailable for sampling. Although I did not sample surrounding soils, my findings support the hypothesis that in colder climates and more northern latitudes such as Canada, these insecticides are persisting in agricultural soils (Goulson, 2013; Bonmatin *et al*., 2015; Jones *et al*., 2014). Even though Prairie soils remain frozen during spring, it is likely that the top few centimeters of soil are thawed and mobilized during the spring melt. The potential for an annual spring "flush" of neonicotinoids to wetlands during early season wetland filling via snowmelt through to the completion of seasonal melt may be creating a long-term, or repeated exposure profile in regional waterbodies.

6.1.3 Ecological and landscape drivers of neonicotinoid fate in Prairie wetlands

Throughout my study, many field-observations prompted the question: why do neonicotinoid concentrations vary so drastically between wetlands that appear both biologically and physically similar? Therefore, a key objective of Chapter 4 was to determine which wetland and landscape features may be most useful to predict neonicotinoid contamination. This research responded to a recognized need to understand neonicotinoid fate in the wetland environment (Anderson et al., 2015; Van der Sluijs *et al*., 2015) and expand the current knowledge surrounding wetland ecotoxicology (Catallo, 1993; Goldsborough and Crumpton, 1998). Numerous variables such as depth, turbidity, surficial plant cover (e.g., floating plants), pH, and

surrounding vegetation have either been speculated to affect neonicotinoids (Morrissey *et al*., 2015) or shown to affect fate and persistence of other pesticides in wetlands (Sarkar *et al*., 1999; Elsaesser *et al*., 2011). In studies of North American water bodies, neonicotinoids have been detected in rivers, streams, wetlands, and even field puddles; however, there is a paucity of data indicating what may be influencing both transport and fate. Perhaps most interesting, the results of my exploratory analysis indicated that of all 59 variables recorded, dominant plant community composition was consistently the most important factor in the models. Shallow marsh plant species identity most explained the greatest variation in both detection probability and neonicotinoid concentration in Prairie wetlands. To my knowledge, no other studies have found presence (or absence) of specific plant species to be a driver or indicator of pesticides in wetlands. This is an important finding as large scale models currently used in pesticide analysis (e.g., soil and water assessment tool (SWAT); Brown and Hollis, 1996), typically ignore the importance of local scale variables such as plant species. Buffer vegetation width in non-Prairie wetlands is often identified as being critical to pesticide mitigation efforts (Lovell and Sullivan, 2006; Carluer *et al*., 2011; Stehle *et al*., 2011), but vegetation composition, not just structure, are important considerations when examining neonicotinoid contamination of Prairie wetlands. The relative importance of species identity, vegetation disturbance or other factors may act as a complex surrogate for buffer width alone which explained less than 1% of model variation. Although studies have identified timely degradation pathways through photolysis and hydrolysis (Guzsvány *et al*., 2006; Lu *et al*., 2015), neither pH nor surficial cover – which should influence both - explained much variation in my models. Interestingly, mean and maximum neonicotinoid concentrations decreased with wetland permanency and depth, further supporting results from Chapter 3 that shallow temporary wetlands are most likely to be affected by neonicotinoids. Although my evaluation of wetland and landscape drivers was still highly detailed, additional collection of adjacent field soil samples may have provided improved understanding of persistence and loading in soils; turbidity measures may have also indicated to what level wetlands are experiencing photolysis (Peña *et al*., 2011). However, rapid assessment systems are designed for short evaluation of numerous wetlands which precluded the ability to collect and analyze even more factors. Some caution is needed in interpreting the results of Chapter 4 as mechanisms and interactions between variables of importance should be tested explicitly to understand how these may be affecting both transport and fate in wetlands, especially during the

summer. Future studies should also attempt to more accurately calculate wetland surface areas and volumes of study wetlands to improve understanding of total pesticide environmental loadings (by mass) beyond concentration data.

6.1.4 The ability of wetland macrophytes to uptake or accumulate neonicotinoids

As dominant wetland plant species identity was the most important variable associated with both neonicotinoid detection probability and concentration (Chapter 4), I selected two common wetland plants (*Typha latifolia* and *Alisma triviale*) for use in an experimental study of neonicotinoid uptake ability by wetland vegetation. I then compared this experiment to a field survey of neonicotinoid concentrations in wetland water of vegetated and unvegetated wetlands. This study addressed the recognized need for data on the ability of margin vegetation to mitigate and/or draw up neonicotinoids from soils and water and improve understanding of neonicotinoid behavior in the environment (Goulson, 2013; Van der Sluijs *et al*., 2015). I hypothesized that as neonicotinoids were developed as a systemic pesticide, macrophytes should both readily uptake active ingredients into their tissues and contain quantifiable amounts of the active ingredient of interest once extracted. Indeed, if wetland plants were able to uptake (and accumulate) neonicotinoids into their tissues, this may further explain why buffer width was relatively unimportant in my previous chapter four model results. In terrestrial plants, field margin vegetation has been shown to accumulate both clothianidin and thiamethoxam (Krupke *et al*., 2012; Pecenka and Lundgren, 2015). Because of the high solubility of thiamethoxam and it being the most widely used neonicotinoid across Prairie Canada (Chapter 2), I selected it as my test compound for the microcosm study. However, recognizing the limitations of the analysis, it appeared that neither test plant species had uptaken quantifiable amounts of the insecticide after one week. In contrast, wetland plants from vegetated ponds situated in canola fields contained detectable neonicotinoid residues partly in species such as *Equisetum*, *Typha*, and *Alisma*. We found no differences in mean water concentrations between wetlands that were unvegetated or surrounded by vegetation ($\beta \pm$ S.E.: -0.49 ± 0.37, $P = 0.19$). Although it explained only 13% of the variance in our model, there was also a significant interaction with vegetation presence and time as average wetland concentrations were lower in those wetlands containing intact vegetation zonation. Minor potential influences of low-level rainfall (<10 mm) were also unlikely to significantly influence concentration peaks. However, given relatively little variation was

explained by plant presence alone, it appears that more detailed assessments of plant composition and other abiotic variables need further exploration (e.g., pH, depth). To date, only two other studies have examined neonicotinoid uptake by an emergent (Mahabali and Spanoghe, 2014) or submerged wetland species (Traisup, 2012) with no field studies available in the literature. Mahabali and Spanoghe (2014) found that 78.9% of imidacloprid was retained in plant tissues while the presence of submerged wetland species greatly increased levels of hydrolysis (Traisup, 2012). It is difficult to identify if my microcosm study design may have been insufficient to determine the true ability of wetland plant uptake. In comparable studies of macrophyte uptake and/or mitigation of other insecticides (e.g., malathion), microcosms were designed to be shielded from the elements and allowed a longer period of pre-experiment equilibrium to reduce stress (Bouldin *et al.*, 2005; Brogan and Relyea, 2013; Mahabali and Spanoghe, 2014). Overall loss of water-borne thiamethoxam through other routes was unexpectedly high in both plant species microcosms, but ability to uptake neonicotinoids could be very dependent on plant species, specific active ingredient, or other biotic/abiotic factors which influence biotransformation of parent active ingredients within plant tissue (Nauen et al., 2003; Ford and Cassida, 2008). Future studies should consider the range of plant species identified in Chapter 4 to uptake neonicotinoids and/or understand their ability to act as a mitigation factor for neonicotinoid transport into wetlands. Equally, new research should better examine the rates of pesticide degradation via the complex interactions between biotic and abiotic variables in Prairie wetlands. Although the outcomes of Chapter 4 led me to explore the ability of plants to accumulate neonicotinoids in Chapter 5, it may have been that macrophytes were simply a surrogate indicator of other variables such as water depth, wetland size, and/or complex hydrologic cycles. Therefore, there is a need to understand whether macrophytes significantly accumulate neonicotinoids, influence runoff potential, or alter conditions associated with enhanced degradation of neonicotinoids in surface waters.

6.2 Research contributions and importance

Prior neonicotinoid research has demonstrated that these insecticides are toxic to many non-target species such as bees and aquatic insects (Blacquiere *et al.*, 2012; Morrissey *et al.*, 2015). Conversely, the fate of these insecticides in the environments upon which they are applied has received very little previous attention. Through development of geospatial mapping of

neonicotinoid distribution and use patterns across the Prairies, in tandem with seasonal wetland sampling over one year, I provide unequivocal evidence that these insecticides are becoming ubiquitous in the Canadian agricultural wetland environment (chapter 2). In other surveys of Prairie waterbodies, insecticides typically have been less detected much less frequently compared to herbicides and concentrations are quite low (Donald and Syrgiannis, 1995; Donald *et al*., 2005). In many cases, studies of water do not consider insecticides in their analysis of water quality or too few samples are collected to develop an accurate projection of safe limits (Environment Canada, 2011). However, the results of my research indicate that many of these newer insecticides (i.e., neonicotinoids) are persisting for longer periods of time in the environments to which they are applied and are more readily found in surface waters. Few studies have attempted a large-scale wetland biomonitoring effort across the Prairies, particularly in Saskatchewan which is the highest user of total pesticides in Canada and a very high wetland density. With the amount of agrochemicals annually applied across this region, my research demonstrates that we not only need better pesticide reporting in Canada (e.g., mass applied, geographic location of use), but additionally should consider annual monitoring of all waterbodies including wetlands.

Although no single crop was identified as most likely to influence neonicotinoid concentrations in wetlands, the current economic importance of canola production and reliance of neonicotinoid-treated seeds may be leading to increased active ingredient accumulation in soils with consequences for aquatic ecosystems draining these soils. As regional wetlands also contained detectable levels of one or more neonicotinoids in all seasons sampled, wetland biota may further be chronically exposed to neonicotinoid movement into surface waters. This is especially true in spring as evidence from this study (chapter 3) not only demonstrates that snowmelt transports neonicotinoids to wetlands which are primarily snow fed, but also that neonicotinoid levels become more concentrated in shallower ponds. My dissertation supports prioritizing toxicological studies of chronic exposure to aquatic communities and an evaluation of mixture toxicity based on the toxic unit approach of active ingredients.

The results of chapter two and three have important implications for wetland ecology and associated aquatic food webs. My research has identified neonicotinoid concentrations to be highest as wetland permanence decreases which is relevant for wetland conservation. In spring

(temporary) and summer (temporary, seasonal) wetlands are critical for both food production and breeding habitat for birds and other wildlife (Davis and Smith, 2001; Mengelkoch *et al.*, 2004), but neonicotinoid levels may be problematic for sustaining the aquatic insect productivity of these habitats. Results of my study clearly indicate that all wetlands are not equal and greater efforts need to be made to conserve early season ponds (Semlitsch and Bodie, 1998) by reducing both physical alteration and/or limiting seeding in drier years. Additionally, the level of contamination detected in these wetlands may be as detrimental as physical drainage if regional wetlands are unable to remain ecologically productive. As aerial insectivorous birds are currently in decline, there could be a link between losses of prey resources due to changes in wetland insect productivity (Hallman *et al*., 2014).

To my knowledge, my study of wetland and landscape features was the first to examine drivers of neonicotinoid fate in wetlands. In my exploratory analysis (chapter 4), buffer width alone was not strongly associated with neonicotinoid detection probability or concentration. Rather, maintaining a high diversity of native plant species may minimize transport and retention in wetlands by slowing particulate movement from surrounding soils. Similarly, the presence of vegetation more strongly reduced neonicotinoid concentrations over time compared to wetlands missing macrophyte communities with some common wetland species accumulating quantifiable levels of these insecticides (chapter 5). Conclusions of other studies of insecticides have identified the importance of vegetation as a critical component to mitigation efforts through alteration of the abiotic environment (Moore *et al.*, 2007; Brogan and Relyea, 2014). However, the relative importance of Prairie wetland plant species to mitigation efforts should be interpreted cautiously. Wetlands are complex ecological systems where other factors not measured such as seasonal rainfall, levels of DOC, and soil-water interactions which influence concentration and detection need further explanation. Importantly, this research has generated many new and exciting hypotheses such as whether plant communities alter wetland conditions to affect neonicotinoid transport or degradation through hydrolysis. Laboratory studies of neonicotinoid active ingredients have found differences in rates of hydrolysis in acidic, neutral, and alkaline solutions (Sarkar *et al*., 1999, Guzsvany *et al*., 2006), but field-level comparisons remain unstudied.

Given the significance of wetland vegetation for buffering aquatic communities from various stressors (e.g., sedimentation, eutrophication) and providing habitat for wetland-dependent organisms, I recommend that maintenance and restoration of Prairie wetland vegetation be a priority for wetland management. The effects may be exasperated in dry years, when many temporary and seasonal wetlands are cultivated for agricultural use which may be leading to formation of persistent sources of neoncotinoid contamination when insecticide residues are solubilized in wet years. Indeed, maintenance of biologically healthy wetland ecosystems is often dependent on the preservation of diverse wetland vegetation which is critical for wetland function (Guntenspergen et al., 2002; Kantrud and Newton, 1996). This thesis also highlights the importance of considering chemical stressors when assessing wetlands and other aquatic systems for overall ecosystem health as this criteria is too often overlooked. Regional wetland policy is often considerate of physical alterations of wetlands, yet chemical stressors such as pesticides may be acting as an equally detrimental form of degradation. However, my dissertation results are likely applicable when considering soluble pesticides entering other aquatic systems in similar agricultural environments.

A central goal of my dissertation was to develop truly interdisciplinary research, specifically by better integration of the disciplines of wetland ecology and ecotoxicology. Through creation of a hybrid wetland assessment and classification system common to wetland ecology (Stewart and Kantrud, 1971; Millar, 1976), my research provides the first model examining numerous (59) wetland and landscape features that influence neonicotinoid detection probability and concentrations in Prairie wetlands as well as overall wetland health. This is especially important for advancing the field of wetland ecotoxicology as studies of other ecosystems often cannot be generalized to predict wetland responses to pollution (Catallo, 1993). Furthermore, Schriever and Liess (2007) have indicated that there are virtually no studies currently available that predict risk of contamination at the landscape level that is validated by field data collection. By developing an ecological model that emphasized the importance of biophysical variables across a range of scales in relation to a chemical stressor (e.g., neonicotinoid insecticide), my results have demonstrated that factors such as vegetation presence may be easily overlooked in traditional chemical surveys. Indeed, the macroecological approach to ecotoxicology has shown that biotic and abiotic factors interacting with landscape

characteristics can significantly alter the effects of contaminants on biological communities (Beketov and Liess, 2012).

With the development of new alternative compounds such as flupyradifurone, there is always the potential that neonicotinoid alternatives may be worse. Additionally, the return to pyrethroids would potentially increase the number of applications needed while insecticides such as organophosphates may increase toxicity concerns to both humans and vertebrate wildlife. However, my results highlight the need to re-evaluate our current farming practices in terms of prophylactic use of seed treatments and more closely examine the behavior of insecticides in the natural environment as many of these newer compounds are more persistent and mobile than originally claimed. It is yet unclear as to whether there will be any other long-term issues surrounding seed-treatment use.

My dissertation demonstrates that utilizing tools from numerous disciplines aids in a more robust study of dynamic ecosystems such as wetlands with results that are potentially applicable, and of interest, to a range of disciplines including wetland ecology, ecotoxicology, agroecology, and policy. By overcoming challenges of interdisciplinary research, my dissertation contributes to a wider examination of pesticides, agroecosystems, and wetlands, while also providing results to tackle complex issues surrounding biological conservation.

6.3 Implications for Prairie wetland sustainability

With the continuous growth in use of neonicotinoid treated-seed across Prairie Canada, adjacent wetlands may either be annually or bi-annually exposed to neonicotinoid contamination based on crop rotation schedules. Indeed, reliance on chemical inputs – including both fertilizers and pesticides – is likely to reduce the ability of wetland ecosystems to provide goods and services (Tilman *et al*., 2002). Agrochemical use patterns may also be the main impassible barrier to expanding adoption of wetland conservation in the Canadian Prairie Pothole Region. Due to landscape simplification across the Great Plains, chemical inputs are only forecasted to grow exponentially in the future (Tilman *et al*., 2001; Meehan *et al*., 2011). Reverting back to five year crop rotations between planting of oil seeds and other cereals or pulses may lessen seed-treatment insecticide inputs into soils, and consequently adjacent wetlands, but is less likely to be an option during the current economic climate. The same can be mentioned of preserving

seasonal waterbodies during drier conditions rather than seeding through them for ease of production which appears to influence persistence and ultimately variation in concentration. As neonicotinoids are expected to persist in colder regions and shown to accumulate in soils over time (Bonmatin *et al.*, 2005; Bonmatin *et al.*, 2014; Jones *et al.*, 2014), Praire wetlands may experience continuous pulse events during spring and summer rains. This is likely to cause problems for secondary production of insects and the wetland-dependent species (e.g., migratory birds, amphibians) that rely on them. Previous research has suggested that annual declines in insectivorous farmland birds is linked to depletion of aquatic insect food resources in regions where imidacloprid concentrations were above 20 ng/L (Hallmann *et al*., 2014). Other recent analyses have also shown that 74% of water studies worldwide exceeded critical chronic toxicity effect thresholds for invertebrates when above 35 ng/L (Morrissey et al 2015). My study was no exception with 51% of the wetlands sampled exceeding this threshold. Moving toward implementation of the Agri-Environment (Tscharntke et al., 2005) approach could be more useful for long-term sustainability as it emphasizes formation of complex landscapes to reduce pesticide use and develop a reliance on natural pest-control measures. Most potential pests can be controlled by natural enemies rather than the numerous specialized chemicals sprayed annually (Tscharntke et al., 2005) and the same is likely to be true of extensive seed-treatment use.

As study wetlands surrounded by economic crops (and some grasslands) contained detectable levels of one or more neonicotinoids in all seasons sampled, wetland biota may further be chronically exposed to neonicotinoid movement into surface waters. This is especially true in spring as evidence from this study not only demonstrates that snowmelt transports neonicotinoids to wetlands which are primarily snow fed, but also that neonicotinoid levels become more concentrated in shallower ponds. Maintaining plant buffer zones in winter is shown to greatly limit the movement of pesticides into waterways (Syversen, 2005), but many landowners remove vegetation to increase crop yield potential, during harvest or before cultivation, especially in drier years. Although our results in Chapter 4 indicated that buffer width was marginally important, the results of Chapter 5 demonstrate that vegetated wetlands situated in agricultural fields have lower neonicotinoid concentrations over time compared to unvegetated wetlands. Further gains appear to be had by improving the diversity of native wetland plants through maintenance of natural wetland zonation. This has important implications for Prairie potholes as

farmers who reduce cropping of the wet meadow and shallow marsh zones in all years may subsequently reduce the amount of neonicotinoid surface and subsurface runoff to area wetlands. Indeed, studies of plant cover have demonstrated that vegetation strips are the most important characteristic influencing retention leading to pesticide degradation via hydrolysis or photolysis (Stehle et. al., 2011).

6.4 Recommendations for future research

Perhaps not surprisingly, this research has led me to ask as many future questions as current answers. Future research should attempt to determine experimental explanations of identified drivers of both detection probability and concentration; it would be useful to understand why certain variables have predictive power. These could include both controlled laboratory mesocosm and field studies where selected variables are isolated and manipulated. This is especially true of the importance of plants where it remains unclear as to whether they are acting as a proxy for complex interactions, providing some level of phytoremediation or perhaps even altering the wetland environment to increase/decrease neonicotinoid persistence. It would be useful to conduct a more comprehensive assessment of both emergent and submerged plant species ability to mitigate neonicotinoids in the wetland environment. This should also necessitate examining whether uptake by wetland plants leads to re-introduction of neonicotinoids to water during senescence later in the season.

Furthermore, additional experimental research into degradation rates in the natural wetland environment and/or the factors that could be influencing overall persistence (e.g., pH, turbidity, DOC, sulfates) would be useful as much of the current research has relied heavily on laboratory studies. The same can be mentioned of rainfall and how it influences neonicotinoid detections and concentrations in Prairie wetlands. In this study, rainfall events were rarely at a level sufficient to influence overland flow, but concentrations varied between years and sites suggesting a need for examining soil-water interactions. In some reference landscapes (i.e., grassland, pasture), wetlands still contained detectable levels of neonicotinoids. Inclusion of surrounding soil samples, nutrients, and better understanding of wetland-groundwater interactions with the extensive wetland assessment criteria may further identify other factors that are influencing both fate and transport in Prairie wetlands.

Although we did not detect neonicotinoid active ingredients in most sediment samples, our sampling technique may have influenced our number of detections. These results should be interpreted cautiously as our limits of detection were much higher than in water and future studies should focus on sampling only the top 1 cm of sediment and investigating the influence of sediment-pore water. In spring, it would be useful to more thoroughly understand neonicotinoid concentrations in agricultural soils and what portion of those soils are able to be mobilized during snowmelt. It is also unclear how neonicotinoids may affect wetland function or whether they behave differently in other regional aquatic systems. To that end, I would suggest using a similar set of criteria outlined in Chapter 4 to investigate whether my set of predictors are broadly applicable to other ecosystems, especially in the Prairies. Perhaps most important is development of long-term monitoring in many of these regions to evaluate changes in water concentrations in space and time and how wetland biota respond to a potentially chronic influx of contamination through ongoing use of seed treatments. This may aid in policy and management decisions regarding conservation of these critical freshwater resources.

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APPENDIX A: SUPPLEMENTAL INFORMATION FOR CHAPTER 4

Table A1: Description of the landscape and wetland feature variables collected as part of field-validated rapid wetland assessment of Prairie wetlands.

Variable	Measure
Total neonicotinoids (ng/L)	Sum of all neonicotinoids
Neonicotinoids: detected/non-detected	Analytical detection
Year	Year of sampling assessment
Landscape setting	
Area	Area in hectares (ha)
Latitude	Latitude
Longitude	Longitude
Previous year's crop	Identified by stubble/rotation schedule
Current crop	Plant identification/rotation schedule
Elevation	Elevation in meters (m)
Closest wetland	Est. distance to closest wetland (m)
Number of visible surrounding wetlands	Count of surrounding wetlands
Surrounding land use	Categorization of land use
Windbreak	Presence/Absence
Wetland connectivity	Presence/Absence
Rainfall	Number of days since last rainfall >10mm
Hydrogeomorphology	
Landscape situation	Description of topographic position
Partially cropped	Wetland planted to crop: Y/N
Adjacent road	Wetland divided by road
Partially drained	Some drainage has occurred
Drainage canal	Wetland has drainage canal
Consolidated	Independent wetlands are consolidated
No alteration	No visible alteration/naturalized
Vegetation removed / bulldozed	Vegetation is/has been removed
Culverted	Culvert between wetlands
Dugout	Wetland is actually a dugout
Hydrology	
Basin fill	% of wetland basin full of water
Wetland depth	Central pond depth (cm)
Dissolved oxygen	DO measured in mg/L
pH	pH
Conductivity	Conductivity
Temperature	Temperature (Celcius)
Standing litter depth	Depth of plant litter (cm)
Bare bottom	Est. visibly devoid of plant community (%)
Algae cover	Est. of algae cover (%)
Sediment description	Sediment color/material
Surface vegetation cover	Est. surficial cover by plants (%)
Wetland classification	
Wetland classification	Based on Stewart & Kantrud (1971)
Vegetation analysis	
Wetland barrier vegetation	Presence/Absence
Wetland barrier: reeds	Presence/Absence
Wetland barrier: rushes	Presence/Absence
Wetland barrier: sedges	Presence/Absence
Wetland barrier: grasses	Presence/Absence
Wetland barrier: shrubs	Presence/Absence
Wetland barrier: trees	Presence/Absence
Actual measure (wetland buffer)	Measured width (m)
Vegetation disturbance (%)	Est. vegetation disturbance (%)
Vegetation cover type	Numeric description based on Millar (1976)
Vegetation Continuity: trees	Visual continuity of vegetation (%)
Vegetation Continuity: shrubs	Visual continuity of vegetation (%)
Vegetation Continuity: reeds/rushes	Visual continuity of vegetation (%)
Vegetation Continuity: grasses/sedges	Visual continuity of vegetation (%)
Average measure: wet meadow	Average measure of four zone points (m)
Average measure: shallow marsh	Average measure of four zone points (m)
Average measure: emergent	Average measure of four zone points (m)
Average measure: open water	Average measure of four zone points (m)
Average measure: trees/shrubs	Average measure of four zone points (m)
Dominant species: emergent	Identity of dominant species
Dominant species: open water	Identity of dominant species
Dominant species: trees/shrubs	Identity of dominant species
Dominant species: wet meadow	Identity of dominant species
Dominant species: shallow marsh	Identity of dominant species

Table A2: Model fit statistics and parameters selected for boosted regression tree models: neonicotinoid analytical detection and concentration.

Model Parameters	
Neonicotinoid analytical detection	
Learning rate	0.002
Bag fraction	0.6
Tree complexity	3
No. of variables	21
AUC	0.987
No. of trees	1500
Deviance explained	62.4%
Neonicotinoid concentration	
Learning rate	0.003
Bag fraction	0.65
Tree complexity	3
No. of variables	23
AUC	n/a
No. of trees	1150
Deviance explained	74.7%

Table A3: Individual dominant shallow marsh and wet meadow plant species identified to influence neonicotinoid detection and concentration in Prairie wetlands. Symbols (+/-) indicate species is associated with a higher (+) or lower (-) likelihood of neonicotinoid detection and concentration.

				Shallow Marsh (SM)		Wet Meadow (WM)	
Sp. Code	**Scientific Name**	**Common Name**	**Native Status:**	**Detection**	**Concentration**	**Detection**	**Concentration**
AGRSMI	*Agropyron smithii*	Western wheatgrass	Native			+	-
AGRTRA	*Agropyron trachycaulum*	Slender wheatgrass	Native	-	-		
AGRSCA	*Agrostis scabra*	Rough bentgrass	Native	+	-	+	-
ALITRI	*Alisma triviale*	Northern water plantain	Native	+	+	+	+
ASTBOR	*Aster borealis*	Northern bog aster	Native	-	**n/a**		
AVEFAT	*Avena fatua*	Wild oat	Non-Native	+	+	+	+
BECSYZ	*Beckmannia syzigachne*	American sloughgrass	Native	+	+	+	-
BROINE	*Bromus inermis*	Smooth brome	Non-Native			-	**n/a**
CALCAN	*Calamagrostis canadensis*	Canada bluejoint	Native	-	**n/a**	+	+
CARAQU	*Carex aquatilis*	Water sedge	Native	-	+		
CARATH	*Carex atherodes*	Wheat sedge	Native	-	-		
CARAUR	*Carex aurea*	Golden sedge	Native			+	-
CARBEB	*Carex bebbii*	Bebb's sedge	Native	-	**n/a**		
CIRARV	*Cirsium arvense*	Canada thistle	Non-Native	-	**n/a**	-	-
ELEERY	*Eleocharis erythropoda*	Bald spikerush	Native	+	+	-	**n/a**
EQUFLU	*Equisetum fluviatile*	Water horsetail	Native	+	+	+	+
EQUHYE	*Equisetum hyemale*	Scouring rush	Native			-	**n/a**
HORJUB	*Hordeum jubatum*	Foxtail barley	Native	-	+	-	+
JUNTOR	*Juncus torreyi*	Torrey's rush	Native			+	-
LEMTUR	*Lemna turionifera*	Turion duckweed	Native	-	-		
MENARV	*Mentha arvensis*	Wild mint	Native	-	-	+	-
PHAARU	*Phalaris arundinacea*	Reed canarygrass	Native	+	-	+	-
PLAMAJ	*Plantago major*	Broadleaf plantain	Non-Native			+	-
POACEA	*Poaceae spp.*	Grass spp.	Unknown	+	-	-	+
POAPRA	*Poa pratensis*	Kentucky bluegrass	Native			+	+
POLCOC	*Polygonum coccineum*	Water smartweed	Native	+	+		
POTNOR	*Potentilla norvegica*	Rough cinquefoil	Native	-	**n/a**		
RUMOCC	*Rumex occidentalis*	Western dock	Native	-	-		
SCHFES	*Scolochloa festucacea*	Common rivergrass	Native	-	**n/a**	-	-
SCIACU	*Scirpus acutus*	Hardstem bulrush	Native	+	-		
SCIPAL	*Scirpus paludosus*	Prairie bulrush	Native	+	-		
SCIVAL	*Scirpus validus*	Softstem bulrush	Native	+	+		
SIUSUA	*Sium suave*	Water parsnip	Native	-	**n/a**		
THLARV	*Thlaspi arvense*	Field pennycress	Non-Native	+	+		
TYPLAT	*Typha latifolia*	Broadleaf cattail	Native	+	+	+	-

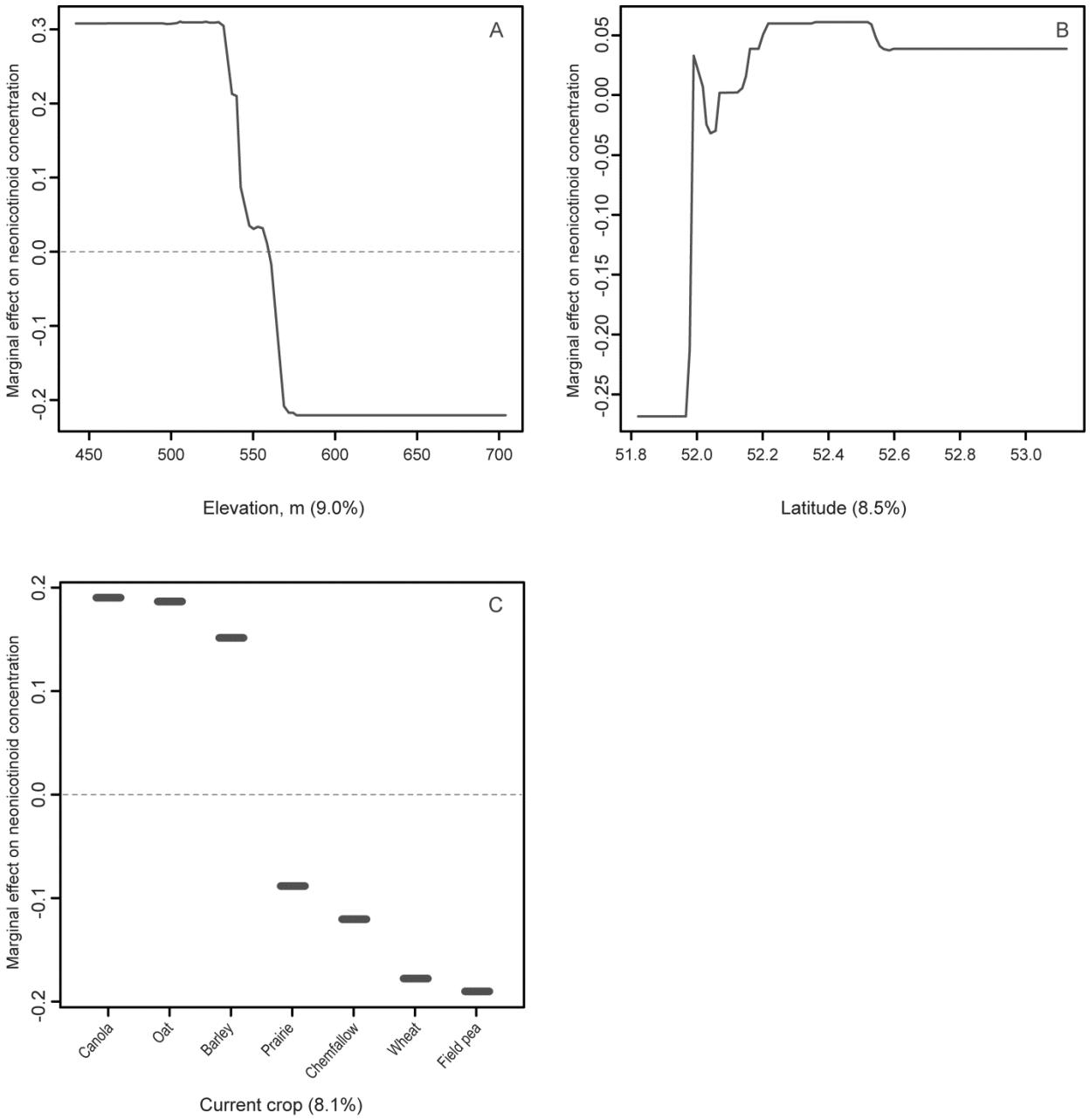

Figure A1-A3: Relationships between concentration of total neonicotinoids in Prairie wetlands and key predictor variables: (A1) elevation, (A2) latitude, (A3) current crop. Details are the same as listed in Figure 4.4.

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Main, A.R., Headley, J.V., Peru, K.M., Michel, N.L., Cessna, A.J. and Morrissey, C.A. (2014). Widespread Use and Frequent Detection of Neonicotinoid Insecticides in Wetlands of Canada's Prairie Pothole Region. *PLOS ONE* 9 (3): e92821. doi: 10.1371/journal.pone.0092821

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Agriculture, Ecosystems and Environment (Elsevier)

Main, A.R., Michel, N.L., Cavallaro, M.C., Headley, J.V., Peru, K.M., and Morrissey, C.A. (2016). Snowmelt transport of neonicotinoid insecticides to Canadian Prairie wetlands. *Agriculture, Ecosystems and Environment* 215, 76-84. doi: 10.1016/j.agee.2015.09.011

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